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THE SOUTH AFRICAN INSTITUTE
FOR MEDICAL RESEARCH.

HANDBOOK OF THE ROUTINE DIVISION.

P. O. Box 1038,
JOHANNESBURG.

THE SOUTH AFRICAN INSTITUTE
FOR MEDICAL RESEARCH.

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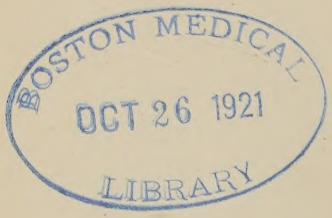

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THE SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH, JOHANNESBURG

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THE SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH.

ENTRANCE FROM EAST COURT

INTRODUCTION.

The South African Institute for Medical Research was established in 1912 by agreement between the Government of the Union of South Africa and the Witwatersrand Native Labour Association (acting on behalf of the Mining Industry of the Transvaal), for the purpose, primarily, of carrying out researches and investigations with a view to the prevention and treatment of human diseases. In addition, the Institute incorporates the old Transvaal Government Bacteriological Laboratory and, in virtue of this, has to perform all those routine public health, medico-legal, and diagnostic services formerly carried out in that Laboratory. Under the terms of its foundation it is also required to carry out many routine investigations for the Mining Industry, more particularly in respect of the health of the great army of Native labourers employed in the mines of the Transvaal.

The Institute occupies a commanding site, in extent just over six acres, adjacent to the Johannesburg Hospital and to the site of the new Medical School, and bounded by Hospital, De Korte, and Joubert Streets, the main entrance (when the full scheme for the laying-out of the grounds has been completed) being designed to face Hospital Street. The site for the buildings, which overlooks the central area of Johannesburg, was donated by the Government, whilst the cost of the buildings and their equipment, amounting to about £42,000, was borne by the Witwatersrand Native Labour Association.

The two foundation-stones, inscribed in Dutch and English respectively, were laid in April, 1913, by Sir Kendal Franks, C.B., M.D., in his capacity as President of the South African Committee of the British Medical Association, and the buildings were

completed in August, 1914. This association of the chief representative of the leading medical organization of the country with the birth of the Institute was of special significance, for apart from the fact that Sir Kendal Franks had taken a prominent part in the negotiations leading to its establishment, the provision of a centre for the communistic activities of the medical profession on the Witwatersrand bulked largely in the minds of the donors of the buildings. This found expression in the provision of a well equipped Lecture Theatre and Library which have been placed freely at the disposal of the profession. Members of the profession visiting Johannesburg from elsewhere will doubtless also find much to interest them in a visit paid to the Institute, and to all such a cordial invitation is extended.

The Institute is controlled by a Board of Management consisting of six members, three of whom are nominated by the Government and three by the Witwatersrand Native Labour Association, the technical direction and control being in the hands of the Director. In addition to the Director the European Staff of the Institute at present numbers 40 persons, and, in the Routine Division, consists of a Superintendent in charge, a Pathologist, and a large body of experienced Technical Assistants.

The work of the Institute is carried on in two main Divisions—" Research " and " Routine." The former is concerned entirely with research work, *i.e.*, the advancement of medical knowledge in matters yet unsolved. With this work the present publication is not immediately concerned, although it may be mentioned, in passing, that the services of the Research Division are always at the disposal of the profession for consultation or advice in any matter in which it can co-operate. The Routine Division, on the other hand, is purely

concerned with the application of already known and settled principles in the diagnosis and treatment of disease, the carrying out of public health, medico-legal, and such-like investigations and the preparation of bacterial vaccines and sera.

Some idea of the progress made by the Routine Division of the Institute since its establishment, and of the important function in medical practice which it discharges, is reflected in the following figures showing the number of routine diagnostic investigations carried out in the last five years :—

Year	1914,	No. of	Routine	Investigations	9,091
,,	1915,	,,	,,	,,	9,804
,,	1916,	,,	,,	,,	15,061
,,	1917,	,,	,,	,,	18,250
,,	1918,	,,	,,	,,	20,866

In the same time the quantity of bacterial vaccines and sera prepared and issued has arisen from very small beginnings to an annual output of approximately 1,000,000 doses. Last year (1918) owing to the demand for the preparation of a vaccine for the prevention and treatment of Epidemic Influenza this output was more than doubled.

Whilst it is generally known throughout South Africa that the Institute performs certain duties for the Government and other public bodies, it is not so generally known that the services of the Institute are available for the carrying out of the ordinary routine laboratory work required by the medical profession. Nevertheless, the exceptional equipment and facilities possessed by the Institute, and the services of its staff of specialists in bacteriology, pathology, and parasitology have been, since its foundation, at the disposal of medical practitioners for the benefit of their patients, although hitherto no special effort has been made to bring these facilities to the notice of the profession generally. The clientele of the Institute is steadily increasing,

however, and is apparently learning to appreciate more the assistance of the "laboratory man" in the diagnosis and treatment of disease. At the same time requests are being continually received for advice as to how and when to send specimens for examination, and for more explicit details as to the use and value of various laboratory products. It is with the purpose of meeting these requests and of thus increasing the usefulness of the Institute to the profession in general that the following notes have been compiled.

These notes fall to be described in three Parts.

(1) When to send specimens for diagnosis, and what to send.

(2) How to collect and send such specimens.

(3) The use of laboratory products in the prevention and treatment of disease.

In Part I.—"Laboratory Diagnosis"—the attempt has been made to deal, in alphabetical order, with all the more common conditions in which a laboratory can be of use in diagnosis. Brief indications are given as to what specimens should be sent for examination, and, in some instances, the interpretation which is to be put upon the reports received is considered in some detail. Naturally, every condition in which laboratory investigation might be of service cannot be mentioned, but a perusal of this section, or of the next, will practically always give some hint as to the sending of specimens from every conceivable kind of case. References are given to the particular page in Part II. where the procedure for collecting the specimen required to be sent is described in detail.

Part II. deals only with the actual methods of collecting and sending of specimens. If the instructions given in this part are followed as closely

as possible, it should result in the material arriving at the Institute in the best possible condition for investigation. Full consideration has been given to the time frequently involved in the transmission of specimens, and in many cases alternative methods are given—one applicable when the specimen can reach the Institute quickly, the other when hours, or days, as the case may be, are necessarily required.

Part III. is concerned with the employment of certain laboratory products—vaccines, sera, etc.—in the prevention and treatment of disease. Throughout Part I. indications are given as to when such products may be useful, but in this section fuller consideration is given to their value, and to the theory and practice governing their dosage, administration, etc.

A Tariff of Fees for Investigations, etc., and Lists of Vaccines and Sera prepared or imported by the Institute, appear as Appendices I. and II. These complete this publication, which, it is hoped, will prove helpful to members of the medical profession as well as of technical service to the conduct of medical practice in South Africa.

THE SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH.

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PART I.

Laboratory Diagnosis.

The position of the laboratory as an aid in the diagnosis, prevention and treatment of disease is now well established, and its function in this respect is more or less standardized.

Diagnosis by laboratory methods has become as much a matter of routine and as essential as the use of a thermometer or a stethoscope; for the information yielded by the laboratory supplies, in an ever-increasing proportion of cases, the indications which are necessary for the proper diagnosis of the disease and treatment of the patient. "Much of this work," in the words of C. H. Browning (opening address, Middlesex Hospital, October, 1918), "has now become routine and it is most unfortunate that it has been frequently entitled 'clinical research.' The name is totally misleading, and no policy more inimical to progress could be conceived than that of many hospitals which make arrangements solely for this class of hackwork." Such routine procedures may, and do, embody the fruit of past clinical research, but research in the future will have to be conducted on a very much wider basis.

The material sent for ordinary examination may undoubtedly provide material for "research," but this is apart from the mere routine investigation, and in the main such work will, in the future, be "team work" by co-operating physicians, surgeons, and clinical pathologists.

It is obvious, however, from the specimens which are sent, or are *not* sent, to the Institute for examination, that there is still a certain amount of misconception on the part of some practitioners as to the function of a medical laboratory, and also as to the extent to which it can be of immediate practical use to them in diagnosis and treatment.

Much of the work of the Routine Division is work that almost every practitioner could do for himself. Many, however, have not the time, and others have not the inclination to carry out the various and sometimes rather tedious procedures involved. Other parts of the work, *per contra,* are only possible with the help of a trained specialist, elaborate apparatus, etc., whilst others require so much time that it is not practicable for the great majority of practitioners to carry them out for themselves.

It should be clearly understood that many of the statements, both in this section and in Parts II. and III. are provisional in character, although dogmatic in form. Medicine is happily a progressive science, but its path of progress is littered with the tombstones of opinions hastily adopted and as quickly discarded. The orthodoxies of to-day may be the heterodoxies of to-morrow, and the information and advice tendered in these pages, although it reflects what we believe to be the best available at the moment, may be recanted to-morrow, despite the possibility of a charge of inconsistency being thereby incurred.

We are fortunately in a position to be able to state simply what we consider to be the truth, and the principle which governs our advice and practice is to give the best, apart from any financial or other consideration.

To be always eager after some new thing, like the Athenians of old, may eventually lead to progress, but, whilst we are always willing to cooperate in trying anything new on the frank understanding that it is an experiment, we regard it as our duty in "routine" work to resist the temptation to recommend any procedure simply because it is new. It behoves us to incorporate the findings of

recent research work, but we will only advise the adoption of new methods as routine procedure in diagnosis or treatment when they have been found to be thoroughly well substantiated and useful.

Conditions in which a Laboratory can be of use in Diagnosis.

ABSCESS.—Usually bacterial in origin, but may be due to chemical or mechanical irritants. Worms, insect larvae, etc., sometimes occur in abscesses. Pus from liver abscesses rarely shows amoebae on first opening, but may do so after several days.

For bacteriological examination the pus should be collected as free from any skin or outside contamination as possible, but at the same time care must be exercised not to allow any antiseptic to become mixed with it.

Send (1) Two thin smears of the pus on glass slides (See p. 81).

(2) Some pus in a sterile tube, bottle, or pipette. These can be obtained from the Institute. From this pus, cultures, and if necessary animal inoculations, can be made (See p. 80).

A swab is not a very satisfactory vehicle on which to transmit pus, and should only be used if the pus cannot be collected by the other methods suggested.

If more than 24 hours are to elapse between the time of collection and the time of arrival at the Institute, tubes of culture media should also be inseminated on the spot and sent along with the pus and slides (See p. 82).

Vaccines prepared from the organisms found in the pus are sometimes of value in treatment.

ACNE.—Due usually either to a *Staphylococcus,* or to the *B. acnes,* or to these organisms in combination.

Paint the surface of an active spot with a little Tincture of Iodine. When it is dry pierce well into the spot with a sterile needle and, if need be, squeeze out some of the "caseous" or purulent matter from deep down.

Send (1) Two thin smears on glass slides (See p. 81),

(2) Some of the matter in a sterile tube for cultivation; or inseminated culture tubes, which should include both surface inoculations and deep stabs—because the *B. acnes* has to be grown anaërobically (See pp. 80 and 82).

Vaccine therapy is usually very satisfactory, and stock compound vaccines are often as successful as autogenous vaccines, sometimes more so (See p. 120).

ANAEMIA.—Anaemias may be (1) primary, *i.e.*, blood diseases, or (2) secondary, *e.g.*, after haemorrhage, or as a consequence of sepsis, Bright's disease, syphilis, malaria, ankylostomiasis, etc., etc.

Simple blood smears (See p. 58) may be fully diagnostic in some cases, and they frequently give valuable indications. Occasionally a more exhaustive examination of the blood is necessary, including total counts of red and white cells, a relative leucocyte count, and the haemoglobin percentage (See p. 67). For interpretation of blood counts see p. 49).

ANKYLOSTOMIASIS. — This disease produces anaemia, which may be of high degree. Eosinophilia is usually present and therefore blood-films may put one on the track, but the final diagnosis rests on the finding of ova, or actual worms, in the faeces (See p. 79).

ANTHRAX. (A) External form—malignant pustule.—Make smears from the serous fluid in and around the pustules, taking them as early as possible in

the suspected case before mixed infection occurs. Make certain of fixing the bacilli by passing the slides several times quickly through a spirit-lamp flame.

If possible, also send some fluid in a small sterile tube or pipette for cultivation or inoculation. This is important because of the occurrence of bacilli indistinguishable from *B. anthracis* by mere microscopical examination.

Take special care to see that none of the fluid is deposited on the outside of the tube or pipette where it might spread anthrax infection, and make certain that the specimen is safely packed for transmission.

If the pustule is being excised send the whole material along, for examination, in a sterile bottle.

(B) Internal form—Woolsorter's disease, etc.—Usually not diagnosed till after death. A small piece of spleen, or some heart-blood, in a sterile bottle is probably the best material in these cases from which to establish a bacteriological diagnosis.

Sera and vaccines in anthrax.—For notes on their value see page 121.

APPENDICITIS.—A polymorph leucocytosis is indicative of the formation of pus, so that a leucocyte count is advisable if the clinical signs are not perfectly clear. An ordinary blood slide is often sufficient to enable one to say roughly whether there is a polymorph increase or not, but it cannot be called an exact method (See pp. 58 and 67).

ASTHMA.—Blood slides usually show a high degree of eosinophilia in true bronchial asthma. This disease is often, if not always, due to a bacterial infection, and the majority of cases can be greatly alleviated, or even completely cured, by vaccines made from organisms obtained from the sputum. It is very important that the sputum sent for examination should have been coughed up from the

bronchi, and not merely material hawked up from the back of the throat (See p. 75). The possibility of the asthmatic condition being due to fibroid phthisis should be excluded by an examination of the sputum for the *B. tuberculosis* (See p. 76).

BACILLURIA.—Urine obtained with precautions to avoid contamination should be sent for the diagnosis of this condition. An ounce or two in a sterile bottle is sufficient (See p. 71).

BLACKWATER FEVER.—Urine should be sent for examination, also blood films with a view to finding whether a malarial infection is present or not (See p. 58).

BILHARZIASIS (Schistosomiasis). — Blood slides *may* show some eosinophilia. In the common urinary form blood is found in the urine, but only intermittently, not constantly. The diagnosis is confirmed by finding the ova in the urine. The usual urinary infection is with *Schistosomum haematobium* in which the eggs have a terminal spine.

Rectal cases are frequently mistaken for dysentery from the presence of blood and pus in the stools. The diagnosis is made by the finding of the eggs in the faeces; in this instance the common infection is with *S. mansoni* in which the ova have lateral spines.

The larval stage of the Bilharzia worm is passed in small freshwater snails. As the snail hosts of South Africa have not yet been fully worked out, snails from pools and streams in the neighbourhood of infected areas would be welcomed by the Research Division for examination. They travel quite well packed in damp grass in a tin.

BOILS.—These are usually due to Staphylococci. A tendency to boils can practically always be cured by a staphylococcal vaccine, either stock polyvalent

or autogenous. Send thin smears of the pus and some of the pus itself in a sterile tube or pipette for cultivation (See pp. 80 and 81).

It is well also to have the urine examined for sugar, as boils are a common feature in diabetes mellitus.

Insect larvae, etc., sometimes emerge from what looks like a common boil. Preserve these for examination in 70 per cent. alcohol, or, better 10 per cent. formalin in water.

BOTULISM.—See Food Poisoning.

CHOLERA.—For diagnosis in suspected cholera cases a little of the "rice-water" stool should be sent in a sterile bottle for cultivation. Specimens should be forwarded with the least possible delay.

COLDS AND CATARRHS.—Catarrhal affections of the respiratory tract may be due to one organism or to several varieties in symbiosis. Vaccine therapy is frequently of great service either as a prophylactic or as a cure. For fuller discussion of this question see p. 124, and for instructions as to sending sputum, nasal discharge, etc., for examination and the preparation of a vaccine see pp. 75 and 77.

CONJUNCTIVITIS.—Discharge may be collected with a sterile wire loop or small swab. Tubes of blood-agar should be inoculated on the spot, and smears made on glass slides, as the organisms responsible for eye-affections are mostly quickly devitalised shortly after removal from their habitat (See pp. 81 and 82).

CYSTITIS.—Frequently bacterial in origin. Urine obtained with aseptic precautions should be sent for examination (See p. 71); at least 2 ozs. should be sent so that a general chemical and microscopic examination can also be made. Vaccines prepared from the patient's own organism are frequently of great service in treatment (See p. 126).

DENGUE.—This disease may exist in the low country of the East Coast. It is probably due to a filterable virus transmitted by *Culicidae*, but there is as yet no known definite method of diagnosis other than by clinical symptoms.

Inoculation into monkeys of blood serum obtained from a patient during the acute stage of the disease might be of value in diagnosis.

DIABETES.—The urine should be examined for the presence of glucose. Acetone, and di-acetic acid should be tested for in advanced cases, their presence being a guide to the degree of acidosis. Treatment should be controlled by periodic quantitative sugar estimations. A few ounces of urine are sufficient for these examinations; it should be sent in a clean bottle, but it need not necessarily be in a sterile one unless a bacteriological examination is also desired.

DIARRHŒA.—Usually temporary and unimportant, but it is often advisable, especially in children, to have the faeces examined so as to exclude the possibility of Dysentery, *q.v.*

DIPHTHERIA.—Take swabs from the membrane or suspected spot. The patient should not have gargled with any antiseptic for some little time before the swabs are taken.

If a piece of actual membrane is removed by the swab a positive diagnosis can sometimes be given from direct examination of smears from the swab, but a negative report from a direct examination is valueless, and, as a general rule, a report is only made on an 18-hour old culture.

When swabs are sent for examination for the *B. diphtheriae* a report as to any other organisms present is only made when it is specifically asked for. Most throat organisms are capable of growing in diphtheritic throat cultures side by side with the

diphtheria bacillus, but in the case of Vincent's angina, for instance, the specific organisms would be missed unless direct films were examined, these organisms not being culturable by ordinary methods.

DUODENAL ULCER.—In such cases an examination of the faeces for occult blood may be of value as an aid in diagnosis. Only a small quantity of faeces need be sent, but for a correct interpretation of results the patient should, for two days previous to collection, have been on a diet containing no meat or green vegetables.

DYSENTERY.—The typical *bacillary* dysentery stool is dirty white in colour streaked with blood, and *jelly like;* in *amoebic* dysentery it is green- or grey-brown in colour, the blood and mucus being more intimately mixed with faecal matter. These distinctive appearances are not always found, however, and in any case they are only typically present during the first day or two of the attack. It is always a sound policy, therefore, to have the stools examined for an exact diagnosis.

Various flagellates, bilharzia, and other conditions may give rise to symptoms closely resembling those of dysentery.

The mucus of a dysenteric stool is the best portion from which to isolate causal organisms, and when such material is sent for examination it should be taken as early as possible in the course of the disease and sent as quickly as possible after being passed, so that amoebae or bacilli may arrive alive (See p. 79).

If the material is to take 24 hours or more in reaching the Institute, in addition to the fresh specimen, a little should be sent mixed with an equal quantity of double strength Gram's Iodine solution (*i.e.,* Iod. 2 grm., Pot. Iod. 4 grm., Water 300 c.c.). This fixes amoebae so that they can

readily be recognised even though dead. Under such circumstances culture tubes should also be inoculated on the spot, as dysentery bacilli are apt to be swamped out by putrefactive organisms. Tubes of special media will be supplied from the Institute on request (See p. 83).

Dysentery vaccines have been used to some extent in prophylaxis, and both vaccines and sera, but especially the latter, in the treatment of bacillary dysentery (See p. 128).

In *amoebic* dysentery it is important to have the stools examined at weekly intervals for at least a month after the termination of a course of emetine treatment in order to make certain that the patient has not become a "carrier" of amoebic cysts. Should he have done so he is a danger to the community, and he also runs the risk of re-developing acute dysentery at any time, or of developing a liver abscess.

After an attack of *bacillary* dysentery patients may also become carriers, harbouring the bacilli, although having no symptoms. It is probable that dysentery is spread largely through the agency of such carriers. In bacillary dysentery also it is, therefore, wise to have an examination made for the presence of *B. dysenteriae* some time after all acute symptoms have subsided.

ENTERIC FEVERS. (Typhoid and Paratyphoid Fevers).—During the first week of these diseases diagnosis is very difficult on clinical grounds alone, but it is, as a general rule, comparatively easily made by blood-culture. This method of diagnosis is really simple and easily performed and should be more employed as a routine measure than it is. The rule in force amongst the British troops in India might well be adopted as a sound practice. "In the case of any

1st week diagnosis.

patient who, for five days, runs a temperature uncontrolled by quinine, and without any obvious cause, do a blood-culture." Practically all enterics can be caught this way and earlier than they can be by a Widal test. Tubes or bottles into which the blood may be inoculated will be supplied on request (See p. 60).

In the second week of the disease the ordinary confirmatory test is an agglutination one — the "Widal reaction." For collecting blood for this test see p. 62. This test is now always made against *B. typhosus*, *B. paratyphosus "A,"* and *B. paratyphosus "B,"* and a "positive Widal" may be taken as definite proof of the presence of the corresponding fever, except when the patient has been previously inoculated or has had an enteric fever before. The Institute practice is to test every serum in dilutions of 1/30, 1/60, and 1/120 against separate emulsions of each of the three organisms, each emulsion containing several strains. Agglutination at 1/30 with one organism only may be taken as suggesting an early infection with that fever, but we like to see agglutination also in the higher dilutions, thus making the diagnosis more certain.

2nd week diagnosis.

Partial or complete agglutination of two, or of all three, organisms is strongly suggestive of fairly recent prophylactic inoculation. In such cases the blood may give positive agglutination reactions for a year or longer, and the only certain method of diagnosis is by early blood culture.

A "negative Widal" unfortunately does not always mean that the diagnosis can be negatived. Although agglutination reactions in these diseases are usually definite about the eighth day, they may not appear till as late as the twentieth, and in some instances they may not develop at all; this is due

either to some individual peculiarity on the part of the patient, or to infection being due to an aberrant strain of organism too widely removed from those against which the serum is tested.

In cases where a "negative Widal" has been reported, but the symptoms still point to an enteric fever, agglutination tests should be repeated and cultures made from the urine or stools (See pp. 71 and 79). It is probably too late at this stage to make blood-cultures worth while attempting.

In the second week of the disease and onwards the bacilli are practically always recoverable from the faeces, and frequently also from the urine, in which they are being excreted.

It is now well recognised that many enteric cases are "contact cases" from carriers, and it would be a sound procedure if the urine and faeces of all convalescents were bacteriologically ascertained to be free from the bacillus before they are discharged as "cured." A search for "carriers" is usually made by agglutination tests in the first place, with urine or faecal cultures as confirmatory tests in suspicious cases. For notes on vaccines in prophylaxis and treatment see p. 130.

Late diagnosis.

FILARIASIS. — **(1) F. medinensis** (Guinea-worm). The female may be found protruding from an opening in the floor of an ulcer, particularly about the lower extremities.

(2) F. bancrofti. Causes elephantiasis, chyluria, lymph-scrotum, etc. Blood slides taken *at night* may show embryos, though these are often not to be found in the blood of elephantiasis cases (See p. 60).

(3) F. perstans. No special symptoms. Embryos may be found in the peripheral blood both during the day and at night.

(4) F. volvulus. Adult worms are found, much tangled, in fibro-cystic tumour-like swellings, especially about the axilla or popliteal space, but sometimes in other parts of the body. Embryos may be found in blood smears.

Adult filarias are very desirable for museum specimens. They are only likely to be found at operation or *post mortem* in lymphatic glands, connective tissues, or in the body cavities. If obtained they should be preserved by dropping into hot 70 per cent. alcohol containing a few drops of glycerine.

FOOD POISONING. (Ptomaine poisoning, Botulism, etc.).—Cases of poisoning by bad food fall mainly into two groups :—

(*a*) Those due to the *B. enteritidis* or other organisms of the Gaertner group. Gaertner meat-poisoning is a true infection—the animal is infective at the time of slaughtering, and the organisms in the interior of the meat may escape destruction unless cooking is very thorough.

The patient is a source of danger as the discharges may spread the infection.

Blood collected in a manner suitable for culture, see p. 60, and faeces, see p. 79, should be submitted for investigation.

(*b*) Those due to the *B. botulinus* or similar organisms. In this case the meat only becomes infected after slaughtering. It may appear quite good and yet be extremely toxic; fortunately these cases are much rarer than those of group (*a*); thorough cooking of the meat protects against botulism. This condition is a toxaemia, not an infection, and the organisms can only be found in the suspected food (meat-pie or canned material, etc.).

In cases where it is doubtful to which group they belong, both suspected food and blood from the patient should be sent for examination.

GASTRIC CONDITIONS.—Chemical examination of gastric contents is frequently of value in distinguishing between gastric ulcer and carcinoma, etc. Either vomited material or material removed by a stomach tube after a special test meal may be sent for examination, the latter, however, is preferable (See p. 89).

Microscopical or bacteriological examination of gastric contents seldom yield information of much diagnostic importance.

GLANDERS.—Man is occasionally infected by the *B. mallei* in either an acute pyaemic form, or as a chronic ulceration with lymphangitis. For diagnosis, pus or scrapings of tissue should be sent in a sterile tube or bottle so that cultures may be made.

GONORRHOEA.—The following recommendations are based partly on those of the (British) Medical Research Committee. See their Special Report Series, No. 19.

Acute cases.

In active acute cases microscopic diagnosis alone is sufficient for a consistently reliable diagnosis. A couple of thin smears of the discharge on slides should be ample for diagnosis. *On no account send swabs*; they are useless, as the pus dries up and the characteristic cocci become unrecognisable.

In the female, swabs may be used for collecting the discharge, but smears from the swabs must be made immediately upon clean glass slides.

In the vulvo-vaginitis of young girls the discharge may be taken direct from the vulva, but in adult female smears of vaginal discharge are useless for diagnosis on account of the abundance of other organisms in the vagina. In such cases two sets of

smears should be made—(1) from the urethral orifice, (2) from the *os uteri*. They should be taken with a wire loop or swab, using a speculum in the latter case so as to avoid vaginal contamination.

Sub-acute and chronic cases.

In sub-acute and chronic cases the diagnosis of gonorrhoea from the examination of smears taken exactly as in acute cases is only occasionally possible. If the discharge is scanty it should be taken early in the morning before the patient has urinated.

Various methods of increasing the amount of discharge in these sub-acute and chronic cases, and of thus bringing to light gonococci from the deeper tissues, may be employed. Of these may be mentioned (1) the taking of some alcohol by the patient the night before the discharge is collected, (2) prostatic massage, (3) injection of 500 million of a stock gonococcal vaccine. The last procedure, in positive cases, will probably also cause a general reaction—slight rise of temperature, malaise, and joint pains, and such a reaction is presumptive, but not conclusive evidence of the presence of a gonococcal infection. The method is not without risk, however, as it may cause focal reactions also and bring to activity an otherwise latent iritis, salpingitis, etc.

The finding of gonococci in sub-acute or chronic cases makes the diagnosis of gonorrhoea certain, but failure to find them in smears by no means proves that they are not still lurking in the tissues.

In such circumstances cultural methods should be resorted to in addition to simple microscopic examination, but this entails personal attendance on the part of the patient at the laboratory, as the gonococcus will not survive transit outside the human body.

Under no circumstances need urine be sent for examination for gonococci. It is a clumsy method; it is useless for cultures; and, if organisms cannot be found in smears of the discharge they are very unlikely to be found in urine.

In gonorrhoeal conjunctivitis the organisms are usually very easily demonstrable in smear preparations, but in other metastatic infections, such as arthritis, they are often very hard to detect, even by culture.

It will be gathered that the conscientious examination of a case of suspected chronic gonorrhoea may involve repeated examinations and be almost impracticable when the patient lives at a distance from a laboratory. A simpler test is, therefore, a great desideratum, and it seems probable that this will be forthcoming by having the patient's blood serum tested for complement fixation, the antigen used, in this instance, being a polyvalent emulsion of gonococci. The complement fixation test is now adopted as a routine method in America, although it has not so far been extensively employed elsewhere.

Vaccine therapy plays some role in the treatment of gonorrhoea. For a fuller discussion on this point see p. 131).

HAY-FEVER.—This condition is caused by the pollen of grasses or of other plants, the cases due to grass pollen being, in most parts of the world, the more numerous. The varieties of pollen which, in South Africa, may be responsible for hay-fever symptoms have not as yet been worked out. It is a comparatively easy matter, however, by means of grass-pollen toxin, which is readily obtainable from several manufacturers, to find out in any individual case whether the patient is susceptible to grass-pollen poisoning or not. Also, if they are found to be susceptible, a grass-pollen vaccine may be

employed as a prophylactic during the winter season, or as a therapeutic measure for actual attacks. The correct dosage is found out by a simple preliminary test.

HYDATID DISEASE.—Due to infection with the larval stage of *Taenia echinococcus*. The cysts are most commonly found in the liver, but may occur in the lung or other situations.

Diagnosis is usually confirmed by the finding of the characteristic rostellum hooklets in the fluid of the cysts.

INFLUENZA.—Pfeiffer's bacillus appears always to be associated with the disease in South Africa, but whether it is the actual cause must still be regarded as *sub judice*. This organism has been consistently found in the lungs, sputum, etc., in acute influenza, sometimes alone, but more frequently associated with other infective micro-organisms. It is occasionally also recoverable from empyemas, abscesses, meningitis, etc., complicating acute influenzal attacks.

Apart from acute influenzal attacks the *B. influenzae* is frequently found, usually in conjunction with other organisms, in sub-acute, chronic, or recurrent catarrhal affections of any part of the respiratory tract.

For diagnosis send sputum, naso-pharyngeal swabs, pus, or material from whatever source is indicated. What is required is material from which the organisms can be grown and isolated in pure culture.

Vaccine therapy, both with pure *B. influenzae* vaccines and with this organism in association with others from respiratory affections, has been employed as a prophylactic and as a curative measure in acute influenza, and in chronic affections in which Pfeiffer's bacillus is found as the, or one of the, infecting agents (See p. 133).

LEPROSY.—Diagnosis dependent on finding *B. leprae* in smears. Scrape a suspected spot or nodule, penetrating the epidermis if it be still intact. Make smears from the serous fluid which exudes.

As the nose is often one of the earliest parts affected smears of nasal discharge, or better still, of scrapings from the nasal septum, should be made in all cases, even where the nose is not obviously affected.

LEUKAEMIA.—See Anaemia.

MALARIA.—Blood films are essential both for diagnosis and for the control of treatment. For diagnosis they should be taken, if possible, before the patient has had any quinine. At least two slides should be taken in each case, and, when quinine has been taken, a " thick film " (See p. 58 *et seq.*) should also be made. But remember, one good slide is worth half-a-dozen bad ones! If the patient has already taken quinine and there is any doubt left about the diagnosis, stop quinine for a couple of days and take fresh slides.

Blood films may be made at any stage of malaria, but for diagnosis pure and simple they are best taken just before an expected rise in temperature. If they can be taken at different phases of a " go of fever " so much the better.

A report of " no parasites found " does not necessarily mean that the patient is free of malaria or that he has not had it. A differential leucocyte count in such cases will, as a rule, show whether there is a present or recently past infection, the large mononuclear leucocytes being increased up to 15 per cent. or over in malarial infections.

It is only after repeated negative examinations at intervals of weeks and months after the patient has had no symptoms and no treatment that a verdict of " cured " can be given with any assurance.

MALTA FEVER. (Mediterranean or Undulant Fever).—For diagnosis blood should be taken for an agglutination test (See p. 62). A definite positive reaction can usually be obtained as early as the fifth day of the disease.

A *M. melitensis* vaccine has been employed in treatment (See p. 134).

MENINGITIS. (Epidemic Cerebro-spinal; Pneumococcal, etc.).—Cerebro-spinal fluid must be obtained by lumbar puncture (See p. 73). Smears should be made on slides and some of the fluid must also be sent in a sterile tube or bottle for culture.

Blood films usually show a marked leucocytosis, and blood-cultures very often yield a growth of the infecting organism, sometimes indeed when none is obtained from the cerebro-spinal fluid.

In searching for meningococcal carriers cultures from the nasopharynx are essential; films are useless as the meningococcus can only be distinguished from other Gram-negative cocci commonly present in that region by cultural and agglutination methods (See p. 77).

Serum treatment of epidemic cerebro-spinal meningitis with a good polyvalent serum is probably the best method of treatment available. Vaccines have been employed to some extent as prophylactics in treatment of carriers, and also in the treatment of acute infections (See p. 134).

PARATYPHOID FEVERS.—See Enteric Fevers.

PLAGUE.—Blood films usually show a marked leucocytosis, but not invariably. Plague bacilli may be seen in blood films, but this is not a practical method for routine diagnosis.

In septicaemic cases blood-culture is the best method to employ for diagnosis (See p. 60).

In bubonic plague, aspirate some fluid from the bubo with a syringe. If there is plenty of fluid

make one or two smears, fixing them well (See p. 81), but in any case send the fluid for cultural purposes, either in the actual syringe, or after ejection into a small sterile tube or bottle.

In pneumonic plague smears of the sputum should be sent and also some of the sputum for culture.

In all forms of plague, but particularly at the commencement of a suspected outbreak, and in pneumonic cases, it is of the utmost importance that the identification of *B. pestis* should be by culture and animal inoculation, because there are bacilli very closely resembling *B. pestis* in appearance, especially in sputum, which cannot be definitely recognised by microscopic examination alone.

Note, that in bubonic plague the bacilli are frequently not to be found after suppuration has set in, so that the earlier a bubo is examined the better.

In *post-mortem* cases spleen smears and smears from haemorrhagic parts of the lungs should be sent, also small portions of either or both of these organs for culture.

In the case of rats, send the whole animal for examination if it can reach the Institute within 24 hours, having first rendered the fleas innocuous by dipping the whole body of the rat in paraffin. Plague bacilli quickly disappear when putrefaction sets in, so that if a longer time must needs elapse in transit spleen smears only should be sent.

A *B. pestis* vaccine is of considerable value as a prophylactic in presence of an epidemic, although the immunity afforded is not of long duration. (See p. 136).

PNEUMONIA AND PNEUMOCOCCAL INFECTIONS.—In cases of pneumonia a bacteriological examination of the sputum is requisite for an exact diagnosis, and it is now becoming of great importance as a guide to specific treatment. It is very

important that the material collected for examination should be actually coughed-up sputum and not merely saliva or naso-pharyngeal secretion (See p. 75). For really exact work, and when consolidation is present, lung-puncture may be done; it is quite simple and practically without risk (See p. 78).

Pneumococci are by far the commonest, although not the only, cause of lobar pneumonia.

Pneumococcal abscesses, meningitis, joint affections, etc., are not uncommon, usually secondary to a pneumonia, but sometimes independent. Pus from such cases will show the presence of pneumococci.

It should be remembered that pneumococci are very frequently present in the healthy mouth and throat, and that their mere presence, unaccompanied by symptoms, does not necessarily signify "pneumonia." Also, they are commonly a factor in the causation of respiratory lesions other than lobar pneumonia, *e.g.*, rhinitis, bronchitis, or bronchopneumonia.

Vaccines and, to a less extent, sera have now an established place both in the prevention and cure of pneumococcal infections, and it is in this connection that an exact bacteriological diagnosis is of such importance in pneumonia and in respiratory catarrhal affections (See p. 136).

PUERPERAL SEPTICAEMIA.—Vaccine therapy has been found to be of great service in many cases. The infection is usually a streptococcal one, and there should be no delay in using a stock puerperal vaccine until an autogenous one can be prepared (See p. 140).

For methods of collecting material for the preparation of an autogenous vaccine (See p. 86).

PYORRHOEA ALVEOLARIS.—May be due to one or more of a variety of organisms. Amoebae are not uncommonly present and emetine treatment was

in vogue for some time, but the amoebae are now generally believed to be non-pathogenic and emetine has gone out of fashion.

Spirochaetes and fusiform bacilli are also common, and for these the local application of Salvarsan may be found the best treatment.

Common bacteria such as Streptococci and *M. catarrhalis* are frequent.

For diagnosis smears should be made from the pus obtained by passing a sterile wire between the teeth and the gum. If a vaccine is desired, tubes should be inoculated with material thus obtained (See p. 82), but smears should always be sent in addition. Vaccine treatment is useless by itself, but may be of value as an adjuvant to dental measures.

RINGWORM.—(See Skin Diseases, p. 35.)

SEPTICAEMIA.—Blood cultures are the only certain method of diagnosis (See p. 60).

SLEEPING SICKNESS (Trypanosomiasis). — The earliest diagnosis can often be made by examination of films of fluid drawn off from enlarged glands (the cervical glands are generally affected). It is important that the needle inserted into the glands should be sterile and absolutely dry, as water in it will so distort the trypanosomes as to make them unrecognisable. The smears of the fluid thus obtained should be made fairly thick, as the trypanosomes are not likely to be very abundant. At the same time several thick blood films should be made and sent for examination (See p. 59).

In doubtful cases, withdrawal of 10 to 20 c.c. of blood from a vein and examination after centrifugalization is advisable, also an injection of blood or an emulsion of an excised gland into susceptible animals. Bottles of citrate solution into which to put the blood for transit can be obtained on request.

An early diagnosis by one or other of these procedures is of the utmost importance, so that treatment can be started early with some hope of ultimate success. Treatment must be controlled by periodic blood examinations.

In later stages of the disease films made from the cerebro-spinal fluid frequently show numerous trypanosomes.

SKIN DISEASES.—Many skin diseases are due to bacteria or parasitic fungi. The conditions are so variable that it is impossible to give brief instructions which will meet all the cases in which a laboratory examination would be profitable.

Vaccine treatment is applicable in some cases of bacterial skin diseases, especially boils, acne, ulcers, *q.v.*

Pus or scrapings from sores will be indicated from some cases. Both smears and material from which cultures can be made should be sent (See pp. 80 and 81), or smears and culture tubes inoculated direct from the patient (See p. 82).

Epithelial scales may be the only material available in other cases. These should, if possible, be obtained by fairly firm scraping with a scalpel, and not by merely removing the already loose surface scales. Forward them either in a small sterile tube, or, if cultivation is not likely to be required, between two glass slides tied firmly together with cotton or string.

Hairs require examination not infrequently, especially in ringworm cases. In this instance, broken stumps, or hairs around the roots of which appear beads of pus, should be selected, pulled out with forceps, and sent in the same manner as described for epithelial scales.

Insect parasites will occasionally be met with and are worthy of investigation. These are best preserved by dropping into a tube containing 70 per cent. alcohol.

"SORE THROATS."—See Tonsillitis.

SYPHILIS.—The following recommendations are based partly on those of the (British) Medical Research Committee. See their Special Report Series, Nos. 14 and 19.

Primary Stage. Diagnosis should, whenever possible, be made at this stage, and, if patients will only come sufficiently early it can be done. It is of the greatest importance, both for the sake of the patient and for the sake of the community, that a conclusive diagnosis should be made as early as possible. "Treatment undertaken in the early primary stage is relatively simple and assured, and is a matter of weeks; after the infection has become generalized, with development of a positive Wassermann reaction or secondary lesions, it is prolonged, uncertain, and a matter of months. It is bad practice to defer diagnosis and treatment until the typical chancre has developed."

Direct proof by finding *T. pallidum*.

"Every localized lesion, however small and atypical, must therefore be suspected, for a simple papule tending to ulcerate may appear within a few days after infection. The rule should be: Examine every such eruption for spirochaetes, and if these are not found at first—try, try, try again."

Diagnosis at this stage is made by the direct observation of the *Treponema pallidum* in the lesion.

The surest method of identification is by dark-ground illumination. This involves personal attendance by the patient at the laboratory. If this be not practicable the next best method may be employed, viz., examination of stained smears. For

this procedure everything depends on the manner in which the material is collected and the slides made. For directions see p. 85. If these are not followed minutely it is sheer waste of time having them examined, but if they are carefully taken according to the instructions given, spirochaetes should be easily recognizable. But, in the case of a negative report, do not be content with one negative finding.

Before syphilis is definitely excluded there should be at least six negative findings, with a week's interval between each, and there should have been no local treatment during this period, nor any general specific anti-syphilitic treatment; a Wassermann test at the end of three months should also be negative.

It is well worth while making persevering efforts to have the diagnosis definitely confirmed, or as nearly as possible excluded, by repeated negative findings at this stage. Practically 100 per cent. of cases can be quickly and completely cured by salvarsan treatment if instituted before the Wassermann reaction has become positive. The interval between the discovery of spirochaetes and the development of the reaction in the patient's serum may be short, but his fate and those of others may depend on the proper utilization of it.

Secondary Stage. In this, syphilis has ceased to be a local infection and has become a general one. The diagnosis may still be made in some cases by the direct observation of spirochaetes, the examination being made exactly as in the case of a primary sore. This is a practical method with condylomata, somewhat less certain with skin rashes, but is not sufficiently reliable with buccal mucous plaques to be used as a standard routine method.

With the generalization of the infection changes occur in the blood serum, changes which can be recognised and which serve as a diagnostic test by making use of the Bordet-Gengou phenomenon, or, as it is commonly miscalled, the Wassermann reaction. This is the test usually employed for diagnosis at this stage. For a further discussion of its value and limitations see below, and for instructions as to collecting blood for it see p. 63.

Indirect proof, "Wassermann" Reaction.

Tertiary Syphilis.—Send blood for Wassermann test.

Congenital Syphilis.—Send blood for Wassermann test.

Syphilis affecting the Nervous System. (Tabes, G.P.I., etc.).—In these cases cerebro-spinal fluid should be submitted for examination as well as blood. It not infrequently happens that the fluid gives a positive Wassermann when that of the blood may be negative. There are also chemical and cytological changes in the fluid whereby a diagnosis of syphilis of the nervous system can be confirmed.

The Bordet-Gengou phenomenon in Syphilis.—(Complement fixation, or as it is commonly called, the WASSERMANN reaction). This phenomenon is dependent on substances or properties which develop in the blood serum as a result of the general infection of the body with spirochaetes. During the early phase of general infection, and similarly during the phase of dying out of the spirochaetes under treatment, there must necessarily be a stage when the reaction is a border-line or doubtful one—the complement-fixing bodies upon which it depends not being present in sufficient amount to give a clear-cut and definite reaction.

A positive reaction develops gradually during the primary stage. It occasionally becomes manifest in

about a fortnight, but it usually takes from 4—6 weeks after infection to become sufficiently definite to be relied upon for diagnosis.

In the secondary stage a positive reaction can be obtained in about 97 per cent. of untreated cases.

In untreated congenitally syphilitic children over one year of age a similarly high percentage of positive results may be expected.

In tertiary syphilis only about 80 per cent. of cases will give definite positive reactions, much depending upon what treatment has been used, and whether the condition is active or latent.

These figures, based upon results given by various writers with experience of large numbers of cases, show that the test may be relied upon in a high percentage of all cases. They also show that, quite apart from any individual errors in technique, it is not an infallible test; it is, however, the best at present available.

The test is admitted, by all workers of experience, not to be a strictly specific one. Possible errors in the interpretation of results fall into three groups:

Limitations of "Wassermann" Test.

(A) *Positive Reactions in Non-syphilitic Cases.*—Positive reactions, though not usually strong ones, are obtained in quite a fair number of cases of leprosy, leishmaniasis, and trypanosomiasis.

A temporary weak positive reaction may also be obtained in malaria; but usually only during the acute phases. Similarly, weakly positive results may be given by scarlatina cases during their early stages.

In yaws, a disease due to a spirochaete closely allied to that of syphilis, a strong positive result is usually obtainable, so that this test is of no value in differentiating between these two diseases.

These anomalies have been confirmed by many observers. They are quite independent of any faulty technique, and can only be regarded as inherent limitations of the method, which have not yet been surmounted.

Provided that the diseases mentioned can be excluded, however, it may safely be affirmed that a definite positive reaction, obtained with a full technique, justifies a diagnosis of syphilitic infection.

(B) *Negative Reactions in Definitely Syphilitic Cases.*—A certain, but comparatively small, proportion of cases of true syphilis entirely fail to develop complement-fixing properties, as may be gathered from the statistics given above. Why this is so, nobody knows, but the fact remains that it is so.

The fact that a syphilitic patient is or has recently been under mercurial treatment may account for a misleading negative result. For this reason all mercurial preparations should have been discontinued for about six weeks before the serum is tested.

(C) *Faulty Technique.*—There are many modifications in the method of performing the test, almost any one of which, worthy of being called a method at all, will give, subject to the exceptions mentioned under A and B, correct results in strongly positive or frankly negative cases.

The test, however, if properly carried out, is far from being a simple one, and the possibilities of errors creeping in through faulty technique are many. It is in connection with weakly positive and doubtful results that it is of the utmost importance that these errors should have been excluded, as far as humanly possible, in order that such results may be correctly interpreted.

The reason why some sera can only be returned as giving a doubtful reaction (very early or almost cured cases), even with perfect technique, has been

already explained. But the fact that different observers, using possibly slightly different methods, have reported varying results on the same specimens, has raised justifiable doubts on the part of many practitioners as to the value of the test.

To get over this difficulty, efforts have been made to have some one method uniformly adopted as the standard method. Since the method was first applied by Wassermann, Neisser, and Bruck for the diagnosis of syphilis, many modifications in detail especially concerning the antigen employed, have been introduced and almost universally accepted, so that the absolutely "original Wassermann test" is now practically never used. There is as yet, however, no general agreement that any one particular modification is undoubtedly *the* best.

The Medical Research Committee in their Special Report Series, No. 14, decline at present to fix on any one method in all its details as being the one which should be adopted as a standard. They lay down certain conditions, however, with which, they consider, any method must comply to be considered satisfactory. They make various recommendations, but specifically state that these are recommended as standards for the time being only, and are to be accepted as liable to periodic revision; and, furthermore, that they will not hesitate to recommend each and every alteration in technique that is proved to be of value.

Without going into unnecessary detail, we will merely state that the present method employed for the test at the Institute fully complies with the Committee's standards. It is that devised by Tayloi (See *The Lancet,* Jan. 5, 1918, p. 19) and further recommended and described by Hewlett in his Manual of Bacteriology, 6th Edition, 1918.

A good routine method of testing for the Bordet-Gengou phenomenon in syphilis is a *sine qua non* for reliable results, but it is just as important that

the method should be strictly adhered to and faithfully carried out. In this connection we can only give our guarantee that the tests, which are now being made daily and on large numbers of sera, are consistently carried out with scrupulous attention to details and rigorous insistence on the observance of all necessary controls. We believe that we have reduced the possibility of any error in technique to a minimum, but should we ourselves, for any reason, not have faith in any result, we will say so, and ask for another specimen rather than send a report which may be fallacious.

Interpretation of Results.—It should be clearly understood that the report in the case of a Wassermann test is merely as to the presence or absence of the reaction. It is not an unqualified answer to the question "Has the patient syphilis or not?" The result must be interpreted in the light of knowledge of the patient's clinical history and manifestations.

Interpretation of "Wassermann" Reactions.

The scale of grading results in use at the Institute is as follows:—"Strongly positive," "Positive," "Weakly positive," "Doubtful," and "Negative." (Further refinement of gradation is to be deprecated; it may sound very exact and scientific, but it really is not so).

The following suggestions are made as to the interpretation of results:—

(1) *Strongly positive* and *Positive* reactions in cases with clinical histories and, or, manifestations suggestive of syphilis, may be taken as definitely diagnostic of syphilis (provided that the diseases mentioned under "A," on p. 39, can be excluded).

(2) *A Strongly positive* reaction even in a case with a doubtful history may, with the same proviso as in (1), be taken as diagnostic.

(3) *A Positive, Weakly positive,* or *Doubtful* reaction should not, from one examination alone, in cases with a doubtful clinical history or clinical manifestations, be taken as establishing a diagnosis of syphilis. Such a case is to be regarded simply as suspicious, and it is recommended that the test should be repeated on another specimen of serum, preferably one collected 5—7 days after a provocative injection of salvarsan (or one of its congeners) This procedure is advised because the earliest effect of salvarsan, etc., on the Wassermann reaction, in a case of syphilis, is to intensify the reaction.

(4) *A Negative* reaction must be accepted with caution, particularly if the history and symptoms are at all suspicious, in view of the statements made on p. 38 *et seq.* When weighing the evidence "for" and "against," a negative report has its value, but it should not be given the position of the decisive factor. History, symptoms, and possible effects of any treatment must all be taken into consideration. Repetition, as suggested under (3), will be advisable in some instances.

(5) In cases where the patient has previously been definitely diagnosed as syphilitic, and the test is required as a guide to further treatment, weakly positive or doubtful reactions may be taken as an indication that further treatment is necessary.

If treatment is successful the reaction should become negative and remain permanently so. *One negative reaction is absolutely of no value whatsoever as a criterion of cure.*

Harrison states (The Diagnosis and Treatment of Venereal Diseases in General Practice, 1918) that much depends on the stage of the disease when treatment was instituted as to when proof of cure may be accepted. It would seem that in a case in which a thorough course had been started before a positive reaction has ever appeared, if it remains

negative for a year after cessation of treatment, even after provocative injections, a cure may be assured. If, though still in the primary stage, the reaction has become positive before the start of treatment, the reaction may be rendered negative and it may remain so for nearly two years, but even then possibly return. If a patient has once relapsed after a given course of treatment, or only commenced treatment when the secondary stage has been reached, no assurance can be given that the reaction will not quickly return to positive, if treatment is suspended for any length of time.

These are the considered opinions of a writer with large experience. They possibly present the question in the least favourable light, but they must be taken as showing that, although cases in which the disease has once obtained a firm hold may be cured, they can only be definitely considered to be so after a long series of negative tests.

The brighter side of the position is the practical certainty that a cure can be guaranteed if a diagnosis is made and intensive treatment started *sufficiently early*.

It is earnestly requested that when a specimen is forwarded for examination brief particulars as to the history, symptoms, and any treatment should also be sent. We are only human, and it becomes dull work examining hundreds of routine specimens of which one knows no details. A knowledge of the circumstances of a case enables us to express an opinion on the meaning of the reaction found, and it is also a very useful guide as to the sensitiveness of our technique in cases of doubtful reaction. It will also enable us very quickly to collect useful statistics. A mere reaction, done with no knowledge of the case, is of value only to the sender of the specimen. A large number of reactions, if properly collated with the histories, would yield valuable information to the community.

SOFT SORE.—The Medical Research Committee find no sufficient evidence that what is clinically known as "soft chancre" or "soft sore" is a specific disease due to any one definite organism. (A small organism known as "Ducrey's bacillus" has hitherto been commonly regarded as the specific cause). The importance of the condition lies more in the exclusion of syphilis than in the positive recognition of Ducrey's or other organism. It is recommended, therefore, that a diagnosis of soft sore be made on clinical grounds, and this only after syphilis has been excluded by observations extending over a period of twelve weeks. This involves (1) examination for spirochaetes, and (2) testing for the Bordet-Gengou phenomenon (Wassermann reaction) towards the end of the period mentioned. (See under syphilis and pp. 63 and 85) for directions as to collecting specimens.

SPIROCHAETOSIS. (Spirillosis or Tick Fever).—Due to infection with the *Spirochaeta duttoni* through the medium of the *Ornithodorus moubata*, or possibly of some allied species of ticks. Blood slides taken during a pyrexial period will usually show spirochaetes, but as they are sometimes rather scanty, several smears should be made (See p. 58). There is very little likelihood of their being found in smears made during the apyrexial intervals, but smears of juice obtained by a liver-puncture during these periods might be positive.

TETANUS.—In looking for *B. tetani*, pus and necrotic or granulation tissue should be scraped with a sterile Volkmann spoon from as deep down as possible in the suspected wound and sent in a sterile bottle without the addition of any antiseptic, etc. Blood specimens are quite valueless.

TONSILLITIS (and "Sore Throats").—Exclusion of diphtheria by cultures from swabs is important (See diphtheria). In recurrent cases, cultures from

swabs taken during an acute exacerbation may be useful for the preparation of an autogenous vaccine, or smears may show the presence of the organisms of Vincent's angina, etc.

TROPICAL ULCER.—Smears of pus from the ulcer make the diagnosis of this particular lesion easy. They will show enormous numbers of spirochaetes and fusiform bacilli and practically nothing else (See p. 81).

TUBERCULOSIS.—In pulmonary tuberculosis the diagnosis is definitely confirmed by the finding of tubercle bacilli in the sputum. There are a few small precautions which should be observed in the collection and sending even of sputum (See p. 76).

Remember that failure to find the *B. tuberculosis* does not disprove the presence of tuberculosis. Acute miliary cases—"galloping consumption"—may run their whole course without the bacillus ever appearing in the sputum. In the more ordinary type of cases they are not found with "closed" lesions, and even in "open" cases every sample of the sputum will not contain tubercle bacilli. Several examinations at intervals are, therefore, advisable if the case remains at all suspicious.

The routine method in use at the Institute for the detection of tubercle bacillis is calculated to find them even if they are very scanty. If they are not found in smears of likely-looking portions of the sputum, a large quantity is digested with antiformin, and the concentrated residue is then examined. Animal inoculation is only done if specifically asked for, and the results of inoculation cannot be given under a month's time.

In tuberculosis of the urinary tract the urine should be collected in as sterile a manner as possible, since in cases where tubercle bacilli are not

found by direct examination of the centrifuged deposit it may be desirable to have a guinea-pig inoculated.

In bone, joint, or visceral lesions, send pus, fluid, or scrapings, collected aseptically and without the addition of any antiseptic.

TYPHOID FEVER.—See Enteric Fevers.

ULCERS.—Due to a variety of causes. Many are bacterial in origin. Smears of the superficial pus, or cultures made therefrom, will often disclose the causative organisms (See pp. 80 to 82). In others, the superficial discharge contains only secondary invaders, the real offenders being found when this is cleaned off (with sterile water or saline solution—but without antiseptics) and smears made from the serous fluid which exudes after an interval of a few minutes. The routine recommended for the detection of spirochaetes is advised in making smears from such cases (See p. 85). Vaccines are not infrequently helpful in treatment (See p. 143).

VELD SORES.—Usually a staphylococcal infection and generally very amenable to vaccine treatment. If in doubt as to diagnosis follow the suggestions made above under ulcers.

WHOOPING COUGH.—In cases where whooping cough is suspected a lymphocytosis usually appears before the whoop develops, and its presence in such circumstances would justify an early presumptive diagnosis.

For collecting blood for a total and differential leucocyte count see pp. 58 and 67.

Bordet's *B. pertussis* is now commonly accepted as the specific cause of whooping cough. A vaccine of this organism has been considerably employed, both as a prophylactic and in treatment (See p. 143).

"WORMS."—(Especially intestinal).

Blood slides often show an eosinophilia.

A sample of faeces (about 1 oz.) is usually required for diagnosis, which may be made either by the finding of ova or of actual worms.

Specimens of worms will be welcomed for museum preservation. For methods of fixation see p. 95.

YAWS.—Remove crusts. Make thin smears from the discharge underneath the crust. Make other smears from the serous discharge obtained after removal of the superficial pus, in the manner recommended for syphilis (See p. 85).

The *Treponema pertenue* is usually to be found in abundance, particularly in the "deep" slides, but also in the "superficial" ones.

The Bordet-Gengou phenomenon (Wassermann reaction) is definitely positive in practically all cases of yaws.

Notes on Interpretation of Blood Counts.

The following data are given as aids in the interpretation of Reports on Blood Examinations :—

Some approximate Blood Norms.

Haemoglobin	90 - 110%
Colour Index = $\frac{\text{Haemoglobin percentage.}}{\text{Erythrocyte percentage.}}$	0.95 - 1.05
No. of Erythrocytes per c.m.m. :	
At sea level—	5,000,000 - 5,500,000
At altitude of the Rand—	6,000,000 - 7,000,000
No. of Leucocytes per c.m.m.	6,000 - 9,000

Relative Abundance of Various Forms of Leucocytes :—

Polymorphonuclears (Polymorphs)	60 - 75%
Large Mononuclears and Transitional Cells	3 - 7%
Lymphocytes	20 - 30%
Eosinophiles	0.5 - 4%
Basophiles (Mast cells)	0.5 - 1%

Some Physiological Variations.

Age.—The above figures apply to adult males. In infants the total leucocyte figure is about 10,000 per c.m.m.; the percentage of polymorphs is about halved, and that of lymphocytes about doubled. The haemoglobin is 70 - 80% and the erythrocytes 4½ - 5 million. The adult proportions are reached about the age of ten or twelve.

Sex.—In women the haemoglobin percentage is a little less than in men, 80 - 100%. The erythrocytes number 4,500,000 to 5,000,000 per c.m.m.

Altitude.—At heights of 2,000 - 4,000 feet the total erythrocyte figures rises from 5,000,000 to 6,000,000 per c.m.m. and from 4,000 - 6,000 feet it rises to between 6,000,000 and 7,000,000 per c.m.m.

Digestion.—A certain degree of leucocytosis occurs in the early stages of digestion, the lymphocytes being mainly affected. Their increase is both absolute in amount and relative to the other varieties of leucocytes. Blood for leucocyte counts should, therefore, not be taken too soon after meals, or, if it must be so taken, the fact should be stated.

Some Pathological Variations.

(*a*) *Animal parasites,* the finding of which in films gives a positive diagnosis of :—

Malaria, Filariasis, Tick fever, Trypanosomiasis.

(*b*) *Bacterial infections,* detectable by blood culture.

Regularly in :—Anthrax, Plague, Enteric fevers, systemic pyogenic infections.

Occasionally in :—Pneumonia, Meningitis, etc.

(*c*) *Erythrocytes increased in number* :—Polycythaemia, chronic heart and lung affections.

(*d*) *Erythrocytes diminished in number.* (1) Colour index considerably above unity, changes in shape and size of erythrocytes, etc. :—Pernicious Anaemia. (2) Colour index well below unity, no great changes in red cells :—Chlorosis. (3) Concomitant changes variable :—other less well defined types of anaemia.

(*e*) *Leucopenia*—Total leucocyte count below normal :—

Enteric Fevers in their later stages when uncomplicated.

Tuberculosis, when uncomplicated.

Malaria, after or between attacks.

(*f*) *Leucocytes enormously increased in number,* up to 100,000 or even 500,000 per c.m.m. :—The Leukaemias (Leucocythaemias). The increase is almost entirely of lymphocytes in Lymphatic Leukaemia; in Myeloid or Splenomedullary Leukaemia large numbers of a type of cell not found in normal blood—the myelocyte—appear in the circulation.

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(*g*) *Leucocytosis* (or, more strictly, polymorphonuclear leucocytosis), being an absolute and relative increase in the number of polymorphs :—

Suppurative or septic processes, *e.g.*, Abscess, Appendicitis, Pneumonia, Plague, Meningitis, Scarlet fever, Small-pox, Liver Abscess.

(*h*) *Lymphocytosis* — Increase of the Lymphocyte elements. Apart from Lymphatic Leukaemia this occurs in :—

Enteric Fevers in their early stages.

Whooping Cough; appearing before the "whoop" develops.

(*i*) *Increase of large mononuclears* :—

Malaria; very typical of actually present or recently past, the percentage going up to 15 or over.

(*j*) *Eosinophilia*—Increase in the number of eosinophile elements :—

Asthma.

Helminthic (worm) infestations.

Some skin diseases, *e.g.*, Pemphigus, Scabies, etc.

THE SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH.

VIEW FROM NORTH-EAST.

PART II.

Hints on the Collecting and Sending of Specimens.

The days are past when the clinical pathologist received specimens as a favour, with instructions what to look for and still stricter orders how he was to look for it. His position is now established as a co-operator in diagnosis, the sphere of his operations being the laboratory study of particular cases of disease. These operations may end in the laboratory, but they must begin at the patient. As it is impracticable for the pathologist, in the majority of instances, to collect the material for examination himself, especially in a country of vast distances like South Africa, the first steps in his work must be taken for him by his colleague—the practitioner in charge of the case. It is important, therefore, that the practitioner should appreciate, to some extent, the pathologist's position, and that he should observe the precautions which the latter finds to be necessary to the successful carrying out of his particular share of the work. Satisfactory results cannot otherwise be expected from such co-operative work, and it is for this reason that the following hints on the collection of specimens for various purposes are given.

Any specimen sent to us will be examined, and the best will be done with the material supplied; but it is a weariness to the flesh and a waste of everyone's time to attempt any elaborate investigation with specimens improperly collected for the examination desired, or when they arrive in a condition so stale as to be useless. The Routine Division desires to be of as much use as possible, but it needs the careful co-operation of the practitioner in the following respects to make it so.

There are three main conditions which must be attended to by the practitioner when sending specimens if the best possible results are to be secured. These conditions are:—

(1) Collect the Specimen with care.

If this be not done, all subsequent proceedings and instructions are practically valueless. The procedures advised hereafter for the particular kind of specimen which is to be collected should be followed as closely as possible. Their apparent complexity need occasion no alarm. They have been stated fully in order to make them perfectly clear, but they are really quite simple. Attention to detail, however, is the secret of success.

(2) State clearly what you want.

Stitt, in his invaluable "Practical Bacteriology, Blood Work, and Parasitology" makes the following remark, which we thoroughly endorse: "There is one point not sufficiently recognised by physicians and that is that a routine examination is not apt to be as carefully conducted as one calling for a specific feature. Without disparaging the value of routine examinations it is a fact that the internist who knows what he wants gets better results from the laboratory man." If the matter is treated casually — the specimen being collected and forwarded anyhow, with the remark, "For general examination," or even without such an illuminating instruction as this (and it is surprising how many such are received!)—the specimen is not likely to be examined with intelligent purpose, for it can hardly be expected that we should carry out every conceivable test on the chance of finding something to please the practitioner. Life is too short, and there are too many other more useful things which have to be attended to.

(3) Send some particulars about the case.

It is not suggested that a clinical history is required in every case in which, for example, the urine is to be examined for albumen, or the sputum for the *B. tuberculosis,* nor, in fact, when it is

requested that some specific feature should be tested for. If some specific test is asked for, it is assumed that the sender knows precisely what he wants and the examination will be proceeded with in full confidence that the interpretation of the laboratory results can safely be left to him. Nevertheless, the laboratory man is only human, and it undoubtedly makes his work more interesting if he is told a little about the issues involved in even the simplest examination. It also makes results more valuable when they can be collated with the clinical features.

In more complex cases it is almost essential that something of the clinical features of the case should be known. A laboratory is not exactly like the puzzle-solving department of a newspaper, though some appear to regard it as such. Data are frequently necessary in order that the best method of investigation may be selected, and in order to interpret the results obtained.

The two instances in which we are most often "left guessing" in this respect are in the sending of pieces of tissue for histological examination, and of sera for a Wassermann test. In both cases it is really important for us to know something of the clinical facts. (See pp. 44 and 89).

General Instructions as to the Sending of Specimens.

(*a*) Seal up or cork all fluid specimens properly. It is astonishing how many samples of blood, pus, etc., are lost by leakage, through lack of attention to this little detail.

(*b*) Pack all specimens securely in a tin or wooden container. The Post Office does not handle each packet with individual attention, and glassware loose

in an envelope or in a flimsy cardboard box cannot be expected always to survive their handling.

(c) Address specimens and letters fully to—

The South African Institute for Medical Research,

P.O. Box 1038,

Hospital Street,

Johannesburg.

It will ensure correct and quick delivery to put the above address in full every time. Where the investigation required is of particular urgency, the specimen, if collected outside Johannesburg, should be sent by "Express Post."

Our telegraphic address is "Bacteria," Johannesburg.

(*d*) If a specimen and the particulars concerning it are sent under separate covers, please do not forget to put some sort of identification label upon or with the specimen. We receive, on the average, over 50 specimens a day, and mistakes may occur if specimens have to be shuffled and arranged in order to fit them to their descriptions.

Outfits for Collection of Specimens.

When apparatus and material wherewith to forward specimens are required, any in the following list will be supplied, *gratis*, on request :—

1. Glass slides—for blood-films, smears of pus, etc.

2. Sterile swabs—for Diphtheria, uterine discharges, etc.

3. Sterile pipettes—for pus, or separated serum for *Wassermann* test.

4. Sterile wide-mouthed bottles—for urine, faeces, sputum, or pieces of tissue.

5. Sterile pipettes for blood for *Widal Tests.*

6. Sterile tubes—for blood for *Wassermann Test*, cerebro-spinal fluid, etc.

7. Outfit for a "*Full blood count,*" *i.e.,* Estimation of Haemoglobin, Total Erythrocytes, Total and Differential Leucocyte count.

8. Outfit for a Total and Differential Leucocyte count only.

9. Outfit for *blood culture,* viz. : 1 ampoule of sterile citrate solution, and 2 bottles of nutrient broth.

10. Outfit for *blood culture* more suitable for suspected *Enteric cases,* viz. : 1 ampoule of sterile citrate, and 2 bottles of sterile bile.

11. Outfit for making cultures in *Dysentery cases,* viz. : 1 sterile swab, 1 sterile wire loop, 1 tube sterile saline, 4 tubes differential medium, also 2 glass slides and 1 tube of "double Gram."

12. Outfit for making cultures in *Acne cases,* viz. : 1 sterile wire spatula, 3 tubes blood agar, 1 tube glucose agar, and 2 glass slides.

13. Outfit for making cultures in *puerperal cases,* viz. : 1 sterile swab, 4 tubes blood-agar, and 2 glass slides.

14. Outfit for making cultures in any other case, viz. : 1 sterile wire loop, 4 tubes of blood-agar, and 2 glass slides.

Should you have occasion to telegraph for any of the above it is sufficient to quote the prefixed number given above and to state the quantity required; *e.g.,* "'Bacteria,' Johannesburg, send two outfits number five," would be interpreted as meaning that you wished the Institute to send you two pipettes for the collection of blood for the Widal test. Or "'Bacteria,' Johannesburg, send three outfits numbers three and six," would be taken as meaning that three sterile tubes with three sterile pipettes were wanted.

Collection of Specimens.
Blood.

Directions for the Preparation of Blood Films for the Detection of Parasites, or for Estimation of the Relative Numbers of the Leucocytes.

A.—Thin Films for Parasites or Differential Leucocyte Counts.

Apparatus required :—

*3 glass slides.

Hare-lip pin, or flat needle.

The one essential for getting good blood smears is freedom from grease.

Polish the slides with a clean cloth and a drop of caustic soda or a little soap and water. Dry them and get rid of the last traces of grease by passing several times through a spirit lamp flame. Allow to cool.

If a spirit lamp is not available, place a small ball of cotton wool on a plate or saucer. Pour spirit on to it and set alight. It will serve the purpose admirably.

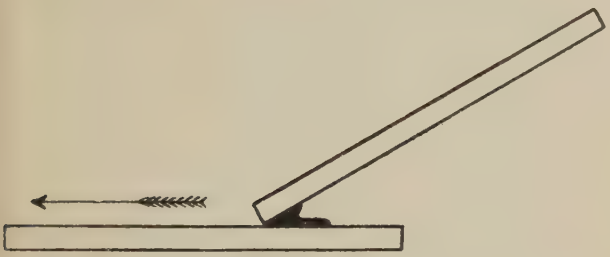
Cleanse the lobe of the patient's ear with soap and water or ether, then dry. Puncture the under edge with the needle or hare-lip pin. If the blood does not flow readily the patient's head should be depressed; as little pressure as possible should be exerted to obtain the blood. Blood may be taken from the finger if preferred.

Wipe away the first drop of blood.

Hold a slide by the long edges between the thumb and the second finger. Apply the under surface of the short edge of the slide to the blood drop. Bring this edge into contact with the surface of a second slide, near one end, so that the blood extends in a line across the slide. Holding the first slide at an angle of about 30°, and pressing the upper surface

*Supplied by the Institute on application.

lightly with the forefinger, push it gently along the entire surface of the second, as per diagram :—



The best films for counts are obtained by slightly chipping the corners of the spreader, so that it is a little narrower than the slide on which the film is spread. This gives two edges to the film at which the leucocytes tend to congregate. A "one edge" film can be made by having the spreader slightly overlapping one side of the slide on which the film is being spread.

By this method the blood is drawn along the surface of the slide, and forms a thin, even, film. Care should be taken that no blood is pushed in front of the spreader. At least two such films should always be made, using a fresh drop of blood and cleaning the slide edge before each operation. Allow the films to dry in the air, protecting them from dust and flies.

Wrap in a sheet of smooth paper so that each slide is completely separated from its neighbours.

Label and pack for transit.

B.—Thick Film Method for Detection of Parasites.

Prepare apparatus and puncture the ear of the patient as directed in Section A.

Wipe away the first drop of blood.

Apply a clean slide to the next two drops of blood, so that a small pool of blood lies on the centre of

the slide. Spread the blood into a circle about the size of a sixpence with a flat needle, endeavouring to get it as evenly distributed as possible. Two such films should be made. Allow the films to dry thoroughly in the air, carefully protecting them from dust and flies. When perfectly dry, label the slides, wrap them up with the films face to face, but with a fold of paper between the ends of the slides so that the films are not actually in contact.'

Notes:—

In acute Malaria cases thin films alone are usually sufficient. In chronic Malaria cases where the parasites are usually scanty, or in cases for the detection of trypanosomes, spirochaetes, or microfilariae, both thick and thin films should be sent.

When examination for Malaria is required, the smears should, if possible, be made before an expected rise in the temperature. It is almost useless to examine the blood of a malarial patient who has taken quinine during the few days previous, or the blood of a Tick-fever case during the apyrexial periods.

Blood films are useless for the determination of the total leucocyte or erythrocyte content of the blood. An approximate idea of whether there is a marked leucocytosis or leucopenia may be given, but no more.

Directions for Making Blood Cultures.

Apparatus required :—

Tourniquet—rubber tubing or bandage.
Large syringe, of 5 or 10 c.c. capacity (serum syringe), with a medium-sized needle.
*2 or 3 special culture bottles.
*Ampoule of sterile citrate solution.
Ether.
Tincture of iodine.

*Supplied by the Institute on application.

Loosen the linen covering on the cap of each culture bottle. Apply the tourniquet to a limb, preferably the arm, select a prominent vein and cleanse the skin over it with ether, and then apply tincture of iodine. The operator's hands should now be cleansed as though for the performance of a surgical operation. Having previously sterilized the syringe by boiling, attach the needle and draw up into the syringe one c.c. of the citrate solution; and, holding the syringe parallel to the long axis of the vein, thrust the needle through the skin in a sloping direction, so that the point enters the vein obliquely.

Gradually draw back the piston of the syringe until the barrel contains at least 5 c.c. of blood.

Release the tourniquet, and withdraw the syringe with needle still attached. Remove the linen covering from a culture-bottle, lightly touch the wax coating on the rubber cap with the flame of a lighted match, push the needle through the rubber cap and express about 2 c.c. of blood into the bottle. Inseminate the remaining bottles in a similar manner.

Mix the contents of each bottle, then seal the puncture in the cap by melting the wax by means of a match flame.

Re-cover the bottle-caps, fill in the necessary particulars on the labels, and return the bottles to the Institute immediately.

A small pad of gauze or wool soaked in collodion may be applied to the skin puncture if necessary.

Notes:—

If a large syringe be not available an ordinary hypodermic syringe may be employed, but less than 2 c.c. of blood does not give cultivation a fair chance.

Citrate solution in the barrel of the syringe is not an essential, but it is a convenience for preventing clotting of the blood.

Directions for Collecting Blood for Agglutination (Widal) Tests, for Enteric, Malta Fever, etc.

Apparatus, etc., required :—

*Special pipette.

Needle.

Spirit lamp, sealing wax or a candle.

Cleanse the patient's finger, or lobe of the ear, with soap and water or ether. Dry. Sterilize a needle, when it is cool prick the part selected with a sharp piano-touch stroke. Blood should exude freely. See that both ends of the pipette are open; apply one end to the drop of blood, which will then enter the stem by capillary attraction. Allow sufficient to enter *almost* to fill the bulb, then gently warm the unused end of the pipette over a flame for a second or two (care being taken to avoid heating the blood) and seal the tip either by fusing the glass in the flame, or by dipping the end into melted candle grease. When the blood has retracted from the used end, owing to the cooling of the air in the portion which was heated, seal it in a similar manner.

Notes:—

Be sure to send enough blood; one drop, which is sometimes sent, is not sufficient. If any difficulty is anticipated in obtaining it, the finger should be constricted with a piece of bandage. There should not be the slightest difficulty in obtaining ample blood, either from the pulp of the finger, or at the root of the nail.

Make certain that the ends of the pipette are thoroughly closed. It is surprising how often this is overlooked, with the result that the serum leaks out in transit and is lost.

*Supplied by the Institute on application.

Directions for Obtaining Blood or Serum for the Wassermann Reaction.

(1) When the Sample is to arrive at the Institute within 24 Hours.

Apparatus, etc., required :—

Tourniquet.
Large hypodermic-syringe needle.
*Special specimen tube.
Ether.
Tincture of iodine.

Apply the tourniquet to a limb, preferably the arm, select a prominent vein, cleanse the skin over it with ether, and then apply tincture of iodine. Having previously sterilized, by boiling, the syringe needle with a wire in its lumen, withdraw the wire, and, holding the needle parallel to the long axis of the vein, thrust it through the skin in a sloping direction so that the point enters the vein obliquely. Allow sufficient blood to escape to fill the small tube to a depth of not less than one inch. When this has occurred, the tourniquet should be relaxed, the needle withdrawn, and a pad of wool moistened with Tincture of Iodine pressed on the puncture. Replace the cork and allow the blood to clot by standing for an hour before despatch. The sample should then be sent to the Institute together with full particulars.

Some prefer to withdraw the blood by means of a syringe. By this method a finer needle can be used, and it is, perhaps, less painful and cleaner. The syringe must be cool and, of course, no citrate solution must be present in the syringe barrel. The blood (not less than 5 c.c.) should be transferred to the specimen tube as quickly as possible.

*Supplied by the Institute on application.

Notes:—

The cork and the tube are sterile, and care should be taken not to infect either of them with extraneous organisms.

The puncture in the skin may be sealed with a tiny pledget of wool soaked in collodion, if necessary.

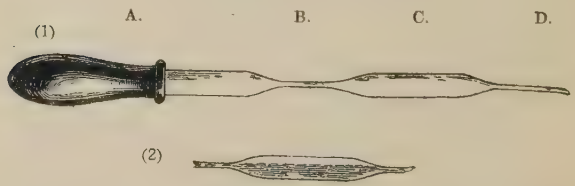
In children, or in persons with very small veins, it is possible to obtain sufficient blood from the pulp of the finger after constriction with a bandage, as described on p. 62. A Widal tube, if practically filled, should afford sufficient serum for the test, but it does not give any too much, and this method of collection is only advisable in cases where the difficulties of venipuncture are very great.

(2) When the Sample cannot arrive within 24 Hours.

If the serum remains too long in contact with the clot after coagulation occurs (and especially if it is being shaken up in the post, etc.), the red blood cells are apt to dissolve in the serum. Such haemolysed serum of itself fixes complement and cannot be depended upon for a reliable reaction. It is strongly advised, therefore, that in such circumstances separated serum should be sent for examination.

Extra apparatus required :—

*Special sterile pipette, as illustrated.



(1) Pipette as issued for use.
(2) Bulb portion C. as sealed up for transmission to the Institute.

*Supplied by the Institute on application.

Inner Court.

Notes:—

Collect the blood as described under Section (1.) Allow the serum to separate from the clot (this usually takes one or two hours — it will separate better if the tube can be kept at blood heat, but this is not essential).

Snap off the sealed end of the pipette at D, slip a rubber teat, or short length of soft rubber tubing, over the plugged end of the pipette at A, and by suction draw the clear serum into the bulb C, taking care not to draw it into the narrow part B. As far as possible avoid drawing up any clot or red blood cells.

Fuse the narrow part of the pipette at B. When it is cool shake the serum to that end and fuse the end D.

If a good flame for fusing be not available, you may seal the end D first by dipping it in melted candle grease, then snap the pipette at B and similarly seal that end.

Pack carefully and despatch with brief particulars of the clinical history, manifestations and treatment.

Serum collected as described should be perfectly sterile and in this condition is suitable for a Wassermann test, even after a week or more, but serum contaminated with micro-organisms is, like haemolysed serum, liable to give fallacious results.

If it is desired to take extra precautions against such risks, and to collect a serum which will keep for months, if need be, one or other of the following procedures may be adopted :—

(*a*) Suck the bulb C only half full with serum, and fill up with pure glycerine. Seal off as before. Mix well by shaking. The amounts of glycerine and of serum should be as nearly as possible equal.

(*b*) After filling the bulb with pure serum and sealing the pipette by fusion, the serum may be "decomplementized" by suspending in water which is kept heated to a temperature of 56° C. (but not over that) for half an hour.

If either of these procedures has been adopted, it should be so stated when sending the specimen.

Directions for the Collection and Dilution of Blood for Haemoglobin, Total Erythrocyte, and Leucocyte Estimations.

Blood-films are required for examination for parasites, or for a differential leucocyte count, but for a total erythrocyte or total leucocyte count a different mode of collecting the blood is necessary. It has not, hitherto, been practicable to have this done except by personal attendance on or by the clinical pathologist, but by the use of a simple apparatus, devised at the Institute, it is now quite easy for a practitioner, situated at a distance, to collect and dilute blood so that upon its receipt at the laboratory, even after a lapse of two or three days, trustworthy counts can be obtained.

Apparatus required :—

* Tube containing special glass capillaries.

* Tubes, marked "E," "L," and "H" containing diluting fluids.

Hare-lip pin or flat needle.

A few matches.

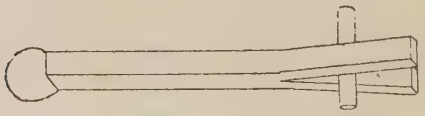
A spirit lamp or candle.

(1) Technique for Erythrocyte Dilution.

Split two matches for about one-third their length from the uncoated end.

*Supplied by the Institute on application.

Extract two glass capillaries from the tube, and fix each in a separate match thus :—



Lay them down upon a sheet of clean paper, close at hand.

Loosen the rubber stopper in the diluting tube marked " E " and stand the tube, in an upright position, in a small tin or jar.

Massage the lobe of the patient's ear slightly, then prick the under surface of it with a hare-lip pin.

Having obtained a large drop of blood by this means, at once take up one of the capillary tubes by its match-holder, and *gently* warm it by passing it fairly rapidly, three times just above the spirit or candle flame. Without further delay, apply one end of the tube to the blood-drop (do not immerse the tube in the blood), holding the capillary in a horizontal position. The blood should now completely fill the capillary.

When this has been accomplished remove the stopper from the tube " E," place the capillary just within this tube and release it from the holder so that it drops directly into the diluting fluid. Replace the stopper and invert the test-tube five or six times, to ensure complete washing out of the capillary. *Leave the capillary in the stoppered tube of fluid.*

One only of the capillaries should be used; the second is supplied merely as a provision against possible accidents.

(2) Technique for Leucocyte Dilution.

Fix three capillaries in match-holders as before. Fill one with blood in the manner described above (1) and place it in the test-tube marked " L." After mixing the contents of the tube, fill a second capillary in a similar manner and drop it also into the tube " L." *Leave the two capillaries in the stoppered tube of fluid.*

Two only of the capillaries should be used; the third is to provide for possible accidents.

For a differential leucocyte count blood films must be made, see p. 58.

(3) Technique for Haemoglobin Estimation.

Fill one capillary with blood—as already described —and drop it into the tube marked " H." Replace the rubber stopper and mix the fluids by shaking.

Notes:—

The capillary tubes are very fragile, and should, therefore, be handled with great care. Forceps may be used for handling them, but the match holders are convenient and less likely to cause breakages.

The diluting fluids are accurately measured volumes; it is important, therefore, that there should be no loss of those fluids.

The technique detailed above should be carried out as quickly as possible in order to prevent the blood clotting in the capillary tubes before it can be washed out by the diluting fluid.

URINE.

Physical and Chemical Examination.

For the purpose of an ordinary simple clinical examination urine does not require to be collected with sterile precautions, but it should at least be

collected in clean utensils and sent as recently passed as possible in a clean bottle. Six or eight ounces will be sufficient.

If it is being sent from a considerable distance it is advisable to check possible putrefactive changes by the addition of formalin, in the proportion of *not more than one drop to each two ounces of urine.*

It is specially desirable that the nature of the examination wished for should be specifically stated. If this is not done, or it is sent merely for "general examination," it should be clearly understood that all that will be reported on is as follows:—

Specific gravity.

Reaction to litmus paper.

Albumen—present or absent.

Glucose—present or absent.

Deposit obtained after centrifugalization, presence or absence of blood, pus, epithelium, crystals, casts or micro-organisms.

If a quantitative estimation of glucose, albumen, urea, etc., is desired, the total quantity of urine passed during a period of 24 hours should be collected and measured; the sample for analysis should then be abstracted from the mixed urine.

Bacteriological Examination.

For a bacteriological investigation of urine it is essential that precautions should be taken in the collection of the specimen to avoid accidental contamination.

It has been abundantly shown that no procedure for obtaining urine from the bladder *per urethram* can be guaranteed not to add urethral organisms to

the specimen, but all other sources of contamination can, and should be, excluded.

In the male, catheterization is rarely necessary; in the female it is the proper procedure.

Directions for Obtaining Samples of Urine for Bacteriological Examination.

Apparatus, etc., required :—

Soap and water.

Methylated spirit.

Cotton wool.

*Sterile wide-mouthed bottle, 1 or 2 oz.

Sterile metal catheter—for female patients.

Male Patients.—Pull back the prepuce. Clean the whole glans and meatus, first with soap and water, then with a pledget of wool soaked in spirit. Allow to dry. While it is drying remove the cap from the bottle and loosen the stopper. The patient should then pass urine, the first portions of which are allowed to escape, only the last ounce, or thereabouts, being passed direct into the sterile bottle. Replace stopper and cap, tying the latter down securely. Label with particulars and despatch without delay.

Female Patients.—Sterilise the catheter by boiling. After analagous measures for cleaning the neighbourhood of the *meatus urinarius,* the catheter must be passed by sight, with every care to prevent contamination through its touching unsterilized surfaces. Similarly discard the first portions of urine, keeping only the last ounce or so.

*Supplied by the Institute on application.

Notes:—

Tubercle bacilli are sometimes found in sufficient numbers in the centrifugalized deposit to make their recognition easy, but they are often scanty, and the most certain method of detecting them is by animal inoculation. This, however, is only done if specially requested.

Gonococci are more readily detected in discharge smears than in urine, and urine is useless for cultivating them from unless the transfer to media is made almost immediately after the specimen is obtained from the patient and the tubes immediately incubated (See p. 82).

Cerebro-Spinal Fluid.

This may be required for chemical, cytological, Wassermann, or bacteriological examination.

Normally the fluid only contains the merest trace of coagulable proteid (globulin). It is usually very definitely increased in inflammatory and syphilitic conditions of the nervous system.

The normal cell content of the fluid is only one or two per c.m.m. In Tabes, G.P.I., Tubercular Meningitis, and Poliomyelitis there is usually a lymphocytosis of the fluid. In Meningitis due to other organisms, meningococci, pneumococci, etc., there is a polymorph and eosinophile leucocytosis.

In syphilitic affections of the central nervous system the cerebro-spinal fluid may give a positive Wassermann reaction, even when the blood has reacted negatively. The converse holds good when the patient is suffering from a general syphilitic infection without the implication of the central nervous system. In a doubtful case both blood and cerebro-spinal fluid should be tested.

The clinical characteristics of all forms of meningitis are so similar that only a bacteriological examination of the fluid can definitely determine the type of infection.

Directions for the Collection of Specimens of Cerebro-Spinal Fluid.

Apparatus, etc., required :—

Large syringe needle, about 1 millimetre bore, and 6 to 8.5 centimetres (2½ to 3½ inches), in length.

*Sterile tube or bottle.

Collodion.

Ether.

Tincture of Iodine.

(1) Sterilise the syringe needle, having a wire in its lumen, by boiling.

(2) Place the patient lying on the left side, with the knees fully flexed upon the abdomen, so that the spine is well arched.

(3) Determine the exact position of the space between the fourth and fifth lumbar spines; this is crossed by a line connecting the highest points of the crests of the iliac bones.

(4) Cleanse the skin over this area with soap and water, then with a little ether, and finally apply tincture of iodine.

(5) The operator's hands should now be cleansed as for a surgical operation.

(6) Take the syringe needle and partly withdraw the wire, so that it does not project beyond the needle point. Hold the needle almost perpendicular to the skin at a point about a quarter of an inch to one side of the median line; thrust it steadily but firmly forward, with a slight inclination inward and upward, so that it pierces the ligamentum subflavum and spinal membranes. As soon as the needle point enters the sub-arachnoid space the fluid should begin to ooze out around the wire.

*Supplied by the Institute on application.

(7) Now withdraw the wire, allow the first few drops of fluid to escape, then collect a specimen directly in the sterile tube, and at once replace the stopper.

(8) Withdraw the needle, and seal the puncture by applying a small pledget of antiseptic gauze soaked in collodion. Rest in the recumbent position for a couple of hours after the operation should be enforced.

(9) The particulars of the case having been duly recorded, the specimen should be sent to the Institute without delay.

Notes:—

As the operation is usually attended by but little pain, and is devoid of serious risk, local anæsthesia is usually unnecessary, except in the case of a very nervous patient.

Should the needle point come in contact with bone the needle must be withdrawn for a short distance, and again pushed forward at a slightly different angle.

If no fluid escape from the needle, it is probably due to one of the three following causes:—

(1) The eye or lumen of the needle has become blocked by a fragment of tissue.

(2) The needle point has not yet pierced the spinal theca.

(3) The purulent fluid is too thick to flow along the needle.

In order to decide which of these causes is operating, first pass the sterile wire along the lumen of the needle; should no fluid now escape when the wire is withdrawn, carefully push the needle a short distance further in. If still unsuccessful withdraw the needle and examine it; should a trace of thick purulent fluid be found to have entered its lumen, it may be expelled on to clean slides, and smears thus prepared for bacterioscopic examination.

should the result still be unsatisfactory a fresh trial should be made, the needle being inserted on the other side of the median line.

On no account attempt to withdraw fluid forcibly by suction with a syringe.

In the case of a child not more than about 5 c.c., and in an adult from 10 to 15 c.c. of the fluid should be removed. It is especially important that these amounts should not be exceeded in cases in which the fluid is evidently under considerable pressure.

SPUTUM.

DIRECTIONS FOR COLLECTING SPUTUM.

A.—For General Bacteriological Examination.

When it is desired to obtain sputum for cultural purposes special precautions must be taken in the collection of the specimen. These precautions are intended to minimise the inevitable contamination with the bacterial flora of the mouth: without these precautions it is impossible to gain accurate information concerning the organisms present in the lower portion of the respiratory tract.

Either of the following procedures may be adopted:—

(1) The sputum—which should consist of material expelled from the chest by coughing, and not merely saliva from the mouth, or material from the back of the throat—should be received in a vessel containing cold, but recently sterilized, water.

The sputum should be gently agitated in the water for a few seconds, and the water then decanted. Fresh sterile water should be at once added, and the sputum again "washed" in this manner. When this process has been carried out three or four times, the sputum—all the water having been finally poured off—should be allowed to slide into a small, sterile, wide-mouthed bottle, which should be immediately closed and sent for examination without delay.

(2) The patient should be instructed to brush his teeth thoroughly with a clean tooth-brush and sterile water. Just before use the tooth-brush should have been immersed for a few seconds in boiling water. No dentifrice, soap, or antiseptic should be employed. The mouth should be well rinsed, the throat gargled four or five times with boiled water, and two or three mouthfuls of it should then be swallowed.

The patient should now cough, and expectorate directly into a sterile, wide-mouthed bottle: the bottle should be immediately closed and sent for examination without delay. No preservative should be added to the specimen.

In cases where it is thought that the patient cannot be trusted to carry out in a sufficiently careful manner the more elaborate technique of the first procedure, the second of these two methods is to be recommended.

If the sputum can reach the Institute within 24 hours it will usually be in quite good condition for growing the organisms present, but if a longer period must elapse it is preferable to inoculate tubes of blood-agar on the spot with sputum washed as described under (1). For general directions as to inoculation of culture tubes see p. 84.

B.—For Examination for the B. Tuberculosis.

When sputum is to be examined only for the presence of the tubercle bacillus the precautions detailed above are not necessary.

The following points, however, call for attention:—

(1) The specimen should be of expectorated material, expelled by coughing, and not merely of saliva.

(2) A specimen collected during an attack of haemoptysis and consisting mainly of blood is of little use.

(3) When there must necessarily be considerable delay before the specimen can reach the Institute, two drops of chloroform should be added to the sputum in the bottle in order to arrest putrefactive changes.

In all cases in which the Bacillus tuberculosis is not found by direct bacterioscopic examination, the specimen is examined by the "Antiformin" concentration method. A biological test—inoculation into guinea-pigs—is not undertaken unless by special request.

Other Morbid Respiratory Fluids.

Nose.

In collecting specimens from the interior of the nose for cultural purposes it is important to use a speculum and to pass the wire loop or swab so that it does not come in contact with the hairs guarding the anterior nares.

If the secretion obtained on a sterile swab can reach the Institute within a few hours of collection it will be sufficient. If a longer time must necessarily elapse blood-agar tubes should be inoculated on the spot and smears of the discharge made (See pp. 81 and 82).

For Leprosy, firm scrapings should be made from the nasal septum, especially from any inflamed or ulcerated spot, and the material thus obtained thinly spread out on glass slides.

Naso-pharynx.

For collecting secretion from the naso-pharynx an ordinary swab may be employed, bending the wire slightly near the swab end (but without touching the sterile swab) and, using a tongue depressor, passing it up behind the soft palate. It is difficult, however, to prevent contamination with mouth organisms, and, also, the swab does not usually reach

far enough. More satisfactory, and equally easily used, is the "West" swab in which the wire and swab are enclosed in a suitably bent glass tube. Remove the plug at the bent end and pass the tube up behind the palate. The swab is then pushed forward slightly out of the tube, given a twist round, withdrawn again into the tube while it is still *in situ,* and the whole is then taken out of the mouth and the plug replaced. The swab may be sent as it is, or cultures and smears made on the spot, depending on the time which it will take for the specimens to reach the Institute.

Pleurae and Lungs.

The fluid from a pleural effusion or empyaema can readily be obtained for a bacteriological examination by aspiration with a sterile syringe and a long, fairly wide-bore needle. The fluid may be transferred to a sterile tube or bottle, or smears and cultures may be made on the spot.

In cases of Lobar Pneumonia when it is desired to obtain the organisms directly from the lungs without the chances of contamination which must always be present with sputum, lung-puncture may be practised without material risk. A moderately fine needle is passed perpendicularly and fairly deeply through one of the intercostal spaces, so that it pierces the consolidated portion of the lung. Some lung-juice is then drawn off with a syringe. As the amount obtainable is usually small it is advisable to have at hand a few culture tubes of blood-agar. Squirt a little of the juice on to the surface of one of them; with a sterile wire loop smear it over the whole surface, then, without doing anything more to the wire, smear it similarly over a second tube. This should give a growth in which the colonies are sufficiently spread out to allow of individual ones being picked off for further investigation.

Faeces.

For a chemical examination for blood, fat, etc., or for a microscopic examination for pus, ova, etc., some 50 grams (2 oz.) of a freshly passed stool should be transferred to a clean bottle and the cork or stopper securely tied down.

For a bacteriological examination the stool should be passed into a receptacle which has been scalded out with boiling water or flamed with methylated spirit. Some 25 grams (1 oz.) should then be transferred with a sterile spoon to a sterile wide-mouthed stoppered bottle and despatched as quickly as possible for examination.

From infants soiled napkins may be sent for examinations.

In Dysentery cases the blood-stained serum or mucus portions of the stool should be specially picked out for examination.

In cases of suspected Typhoid (acute, convalescent, or carrier) a saline purgative is advisable, some of the resulting liquid or semi-solid evacuation being then collected.

It is important that the faeces should reach the Institute as soon as possible after they have been passed. If it is impossible for them to arrive within 24 hours, then, in addition to sending material in its original state, some should be mixed with an equal amount of " double Gram's solution " as advised on p. 21, if examination for Amoebae is desired. For cultivation of dysentery or typhoid bacilli culture tubes of special media should be inoculated on the spot (See p. 83).

Pus, Exudates, Etc.

Directions for the Collection of Specimens of Pus, Exudates, Etc., for Bacteriological Examination.

Special pipette.

This pipette, which is the same as that used for the collection of serum for the Wassermann test (see p. 64) is the most suitable means of forwarding liquid pus, fluids from vesicles, etc.

Before filling the pipette, its narrow end should be broken off close to the tip, and the broken end passed three times fairly rapidly through a candle or spirit flame.

It is most important that the specimen should not be contaminated by extraneous organisms, and it is also important that no trace of antiseptic solution should have been mixed with it.

When the fluid is to be collected from an abscess, sinus, or vesicle, the surrounding skin should first be shaved if necessary, and then cleansed thoroughly with soap and water, and finally with water which has been sterilized by boiling. The pus, serous fluid, etc., a sample of which is required, having been allowed to escape on to the cleansed skin, the point of the pipette should be carefully immersed in it, and the fluid drawn up into the pipette, either by direct suction at the plugged end, or with the assistance of a short piece of soft rubber tubing or teat attached to this end. When the bulb of the pipette is about half-filled fuse the narrow portion of the pipette above the bulb. When cool shake the contents to that end and fuse at the other.

If a good flame for fusing be not available, the point of the pipette may be sealed first by dipping it in candle grease, then snap the pipette at the narrow part above the bulb and similarly seal.

If the pus be too thick to run into the pipette, a specimen may be collected on a sterile swab.

Notes:—

This pipette is *not suitable* for the collection of specimens of blood, cerebro-spinal fluid, urine, or pus in which the presence of the gonococcus is suspected. In the case of the three first mentioned, the proper methods of collection of specimens will be found described in the notes headed "Blood," "Cerebro-spinal Fluid," and "Urine." For examination of pus in which the presence of the Gonococcus is suspected, thin films of the pus (spread like blood-films, see below, on slides) should be prepared and sent for examination.

If the pus or fluid cannot reach the Institute within 24 hours of collection, smears and cultures inoculated directly from the patient should be sent for examination (See p. 82).

Directions for Making Smears of Pus, or Other Fluids, for Bacterioscopic Examination

To make good films for staining, the one essential is clean, grease-free glass slides. To get these, polish the slides with a clean cloth and a drop of caustic potash or a little soap and water; finish off by burning the surface of the slides for a moment in a spirit flame.

When cool, apply a small drop of the pus, or other fluid, about half-an-inch from one end of the slide. At once, with a needle, or with the end of another slide, in the manner described for making blood-films (See p. 58) smears it in a thin film over the greater part of the slide. Do not be afraid of making it too thin; errors in the other direction are more common.

Then fix the preparation by passing it several times quickly through a spirit flame, the film side being towards the flame. Another good method of fixing slides is to drop a few drops of spirit on the

slide, allow it to run over the entire surface, shake off any excess, then apply a light to the remaining film of spirit and allow it to burn itself out.

At least two slides should be prepared in the above manner and sent from each case. After fixation, wrap the slides up separately in a piece of clean soft paper, label with particulars and despatch.

Notes:—

This procedure is specially applicable for the diagnosis of gonococci, *B. leprae,* or other organisms for which cultural methods are not available.

In addition, smears made direct from the patient should be sent in every case in which inoculated tubes are sent, so that the organisms which grow can be compared with those visible in the material originally.

If smears of sputum are being made pick out specially the yellow purulent portions.

Directions for Inoculating Culture-Media with Pus, Faeces, Sputum, Etc.

(1) Pus, Discharge, etc.

Apparatus, etc., required :—

*Tubes of blood-agar.
*Sterile wire loop.
Antiseptic solution.
Boiled water.

Wash the skin surrounding the pustule, wound, sinus, or orifice with antiseptic solution, then remove all traces of the antiseptic with cool, recently boiled, water. In the case of a pustule the contents should now be squeezed out (through a small incision if necessary) on to the cleansed surface of the skin.

Immerse the sterile wire loop in the discharge and lightly smear the material thus obtained over as much as possible of the surface of the medium contained in one of the culture-tubes. Take care not to break the surface of the medium.

*Supplied by the Institute on application.

Inoculate the remaining tubes in a similar manner with fresh loopfuls of the discharge.

In Acne cases, in addition to inoculating one or more slope tubes, a deep tube of glucose-agar should be inoculated by a stab right down through the centre of the medium. The reason for this is that the *B. acnes* will only grow by anaerobic methods. A straight wire spatula is used in this instance instead of a loop, which would split the medium too much.

(2) Faeces.

Apparatus required :—

*Sterile swab.

*Sterile wire loop.

*Tubes of sterile salt solution.

*Tubes of differential culture-medium.

Dip the sterile swab into the freshly passed stool and transfer the adherent material to the tube containing salt solution. Rotate the swab until most of the faecal matter has washed off. Return the swab to its case. Now withdraw the wire loop from the tube and with it take up a droplet of the salt solution in which the faeces have been diluted. Rub the loop lightly over as much as possible of the surface of the medium contained in one of the culture-tubes, taking care not to break the surface of the medium, then, *without recharging the loop,* pass it successively over the surface of the medium in the remaining tubes. The object of this procedure is to secure a growth of discrete colonies, from which individual ones can be picked off for further investigation.

In cases of Dysentery (bacillary) the most favourable material from which to inoculate is the blood-stained serum which is frequently passed at the outset of the attack. In later cases of the disease the

*Supplied by the Institute on application.

best results are secured if some of the mucus be picked out on the wire loop, gently washed in the sterile salt solution, and then used to inoculate the culture-media in the manner described.

In the case of a suspected typhoid carrier, it is advisable to administer a purgative and to make use of the liquid or semi-solid portion of the resulting evacuation for dilution and subsequent inoculation of the culture tubes.

(3) Sputum.

Apparatus required :—

*Tubes of blood-agar.

*Sterile wire loop.

Sterile bowl and supply of cold sterile water.

The sputum should be collected and washed in the manner described on p. 75.

With the sterile wire loop transfer a little of the washed sputum to one of the culture-tubes and lightly spread it over the entire surface of the medium.

Without recharging the loop, proceed to inseminate the remaining tubes in a similar manner.

Notes:—

(1) The cotton-wool plugs of the culture-tubes are usually waxed; the plugs should be loosened in the tubes by softening the wax coating in the flame of a spirit lamp or candle. When the wax has softened, the plugs should be slightly withdrawn in readiness for the subsequent procedure.

(2) The plugs should not be entirely removed from the tubes until the moment of insemination, and should be replaced immediately the operation is concluded.

(3) The portion of the plug which enters the tube should not be allowed to come into contact with the hand or any unsterile surface.

*Supplied by the Institute on application.

(4) All the tubes should be carefully repacked at once and returned to the Institute *without delay.*

(5) In cold weather, before inseminating the tubes, it is advisable to stand them in a warm place—*e.g.*, on the shelf over a stove—for an hour or two; the temperature must not, however, exceed that of the body.

(6) If, during the winter season, an interval of more than two hours must necessarily elapse before the departure of the train, the package containing the inoculated tubes should be kept warm until the moment of despatch.

(7) Tubes of culture-media should not be kept in stock, as after a few weeks, at most, they become dry and useless for the purpose for which they are intended. They should be ordered fresh, by wire if necessary, each time when required.

Directions for Making Films for Examination for the Spirochaete of Syphilis.

Apparatus, etc., required :—

*6 glass slides.

Sharp needle, scarifier, or bistoury.

Cotton wool.

Cool boiled water.

Preliminary Considerations.

(1) Every chancre should be examined for the spirochaete of Syphilis, but,

(2) It is useless examining those which have already been treated with iodoform, calomel, black wash, etc. If any such treatment has been instituted stop it for three days, applying only sterile water dressings or inert absorbent powders such as talc, and then proceed to examine.

*Supplied by the Institute on application.

(3) Make the glass slides grease-free by polishing with a clean cloth and a drop of caustic potash or with soap and water; finally burning their surface in a spirit flame.

Technique:—

(1) If there are several ulcerations select the most suspicious, and, of that, pick out the edge least indurated.

(2) With a pledget of wool dipped in sterile water clean off all superficial pus, etc.

(3) Dry with fresh cotton wool.

(4) With the needle, scarifier, or bistoury make several short close parallel scarifications, crossing both the ulcer and its skin edge at the point selected. These should be just deep enough to draw blood.

(5) If, at first, pure blood flows, arrest it by slight compression with a tampon of wool and wait a few moments.

(6) Allow serous fluid to exude from the scarifications (this takes a minute or two). With the point of the instrument used transfer a small drop to a glass slide and smear out in a thin film. A little blood in the film does not matter, but there should be as little as possible.

(7) Make six such slides. Dry them by waving in the air but do not fix in any other way. Wrap up in clean soft paper so that each slide is separate from its neighbours. Label with particulars and send off for examination.

Puerperal Septicaemia.

Directions for Obtaining Material, or for Inoculating Culture-Media, for the Preparation of an Autogenous Vaccine.

Preliminary Considerations.

When the presence of a puerperal septicæmic infection is suspected it is not advisable that there should be any delay in adopting vaccine treatment.

An autogenous vaccine should be obtained for every case, but pending its completion, which will take some days, one or more doses of a Stock Puerperal Vaccine should be administered. As a routine measure, in order to save delay, we send a few doses of Stock Puerperal Vaccine in every case where Laboratory examination of the specimen shows a positive result. Should any of this be unused when the autogenous vaccine arrives it may be returned.

In cases in which the perineum has been lacerated during the confinement the septicæmic symptoms may be due to infection of this wound. In such circumstances a specimen should be taken from the raw surface of the laceration as well as one from the cervical canal.

No antiseptic douche or application should have been used for some hour or so before the specimen is taken.

A.—When the Specimen can be delivered at the Institute within 4 hours of its collection.

Apparatus required :—

*Sterile swab in case.

Speculum, etc.

In order to obtain the infecting organism in pure culture it is necessary to contaminate a sterile swab by inserting it into the cervical canal. To ensure that the swab shall not come in contact with any other surface a sterile speculum should be passed; with good artificial illumination it is then possible to introduce the swab into the *os uteri* without its becoming contaminated with anything but cervical discharge.

Having soiled the sterile swab with the lochia in this manner it should be replaced in its case and despatched without any delay to the Institute.

*Supplied by the Institute on application.

B.—When the Specimen cannot reach the Institute until 4 hours or more after its collection.

Apparatus required :—
*Sterile swab in case.
*Tubes of blood agar.
*Glass slides.
Speculum, etc.

A specimen having been obtained in the manner already described (A), the contaminated swab should be lightly rubbed over as much as possible of the surface of the medium contained in one of the tubes. Take care not to break the surface of the medium.

Without recharging the swab use it to inoculate the remaining tubes in a similar manner.

See general notes above on the technique of inoculating tubes.

Directions for the Forwarding of Specimens of Morbid Tissues for Histological Examination.

The specimen should include—whenever possible—not only the morbid tissue, but also its junction with the adjacent normal tissue.

If it can reach the Institute within an hour or two it may be sent fresh, and in this case whole organs or large pieces of tissue may be sent.

If longer time must necessarily elapse, representative portions of the tissue, preferably not larger than one inch square by half an inch thick should be sent, and placed in a wide-mouthed bottle containing some fixative.

The volume of the fixative solution should be at least three times that of the tissue put in it, and the bottle should be quite full so as to avoid damage by shaking.

* Supplied by the Institute on application.

As fixative use either

(*a*) A 10% formalin solution (say, one drachm formalin to each ounce of water), or

(*b*) 30% alcohol (say, methylated spirit 1 part, water 2 parts).

Do not cram tissue into a narrow-mouthed bottle, it hardens in the fixative and it is almost impossible to get it out without damage.

Cork tightly and pack securely.

The following particulars of the case should be supplied :—

The patient's name, sex, age, race, and occupation.

Exact anatomical site from which specimen is taken.

Duration of condition.

Provisional diagnosis.

If the specimen be one of new-growth, the colour of the hair and eyes, also the character of the complexion should be noted.

Any other information about the case, which is thought to be of importance or of special interest, should also be added.

Directions for the Administration and Collection of Ewald's Test Meal.

At 7.0 a.m., upon an empty stomach, give the patient 1½ ounces of dry bread or toast and 10 ounces of weak tea, telling him to eat the bread slowly and to sip the tea. At 8.0 a.m. the patient, having received no other food or drink, should assume the recumbent position without pillows. A No. 9 soft rubber catheter is taken and a mark made upon it about 18 inches from the blind end; this latter end is freely lubricated with glycerin.

The patient's tongue, covered with a cloth, is grasped by the operator's left hand and gently withdrawn. The catheter is now taken in the right hand

and carefully passed along the dorsum and over the base of the tongue between the pillars of the fauces; the patient at the same time being directed to swallow.

The stomach is reached when the mark on the tube is opposite the incisor teeth.

By means of gentle pressure applied over the epigastrium with the hand, the contents of the stomach may now be caused to escape along the tube into a clean vessel. If the outer end of the catheter is at a lower level than the end in the stomach a syphoning action is set up. The vessel should be closed and despatched to the Institute, together with the necessary particulars, without delay.

Water.

Directions for the Collection of Specimens of Water for Bacteriological Examination.

In submitting specimens of water for bacteriological examination it is essential that means be taken to keep the temperature as nearly as possible at zero, in order to prevent the organisms from multiplying in transit. For this purpose a special box is issued by the Institute containing an ice chamber.

To Prepare the Box.

(This should be done just before collecting.)

Unscrew the nuts and take off the copper lid.

Take out the inner copper case.

Pack the outer compartments of the larger case with finely chopped ice. In warm weather use a mixture of two parts ice and one part common salt.

Replace the inner box and proceed.

To Collect the Sample.

(1) *From a Tap, or Pump.*

Allow the water to flow for two or three minutes.

Wash the mouth of the tap—inside and out—with the thumb and the finger.

Allow the water to run for another minute.

Take off the cover of the bottle, and remove the stopper.

Do not allow the ground portion of the stopper to touch the hand, and on no account lay the stopper down.

Fill the bottle *almost* full, replace the stopper, giving it a twist into place, and refit the cap.

(2) *From a Stream, Reservoir, etc.*

Having removed the cover, hold the bottle about a foot beneath the surface of the water.

Take the stopper out whilst the bottle is under water, allow the bottle to fill, and replace the stopper.

Lift out the bottle and wash off any scum that may be adhering to it.

Refit the cap.

(3) *From a Bucket-Well.*

Thoroughly clean the bucket—inside and out—with soap and water, then rinse it five or six times in clean water.

Lower the bucket into the well.

Discard the first bucketful of water.

From the second, collect a specimen as in (2).

Label the bottle or its cap with a distinctive mark then pack it carefully in the inner box and refix the lid.

Particulars relating to the specimen should be sent separately on a form supplied with the ice-box.

The routine bacteriological examination of water includes an estimation of the total content of organisms growing at blood-heat, and the determination of the presence or absence of *B. coli* in varying quantities of the sample.

A search for other specific organisms is only undertaken by special request, and it may just be mentioned that isolation of the *B. typhosus* from water supplies is like looking for the proverbial needle in a haystack, and is now very rarely attempted.

The examination of mineral waters is substantially the same as for ordinary waters. At least two bottles of each kind should be sent for investigation.

For chemical examination of waters (which is not done at the Institute, but by the Government Analyst) not less than a Winchester quart sample should be sent.

An estimation of the total number of organisms per c.c. in a water is not reliable when the specimen is collected more than 24 hours before the examination can be started. Even if kept thoroughly well chilled the bacterial content may change considerably quantitatively, and the results obtained are not a true index of the quality of the water *in situ*.

For places at a distance.

When waters take more than 24 hours to reach the Institute it is only practicable to have one test made, viz., that for the presence or absence of *B. coli* in varying quantities. Fortunately this is the most valuable single test in indicating the degree, if any, of faecal pollution. A special outfit has been devised at the Institute to meet the necessities of such cases. With it the necessary dilutions are readily made on the spot, and the samples can be forwarded to the Institute for examination without the use of ice-packing. Full particulars and outfits will be supplied on request, but it should be clearly understood that this "single test" examination is regarded only as a second-best one, and is not recommended except in those cases when, owing to distance from the Institute, the full examination is impracticable.

Milk.

The collection of milk samples for bacteriological examination must be conducted with great care, otherwise the interpretation of the results obtained may be very fallacious.

When the milk of a single cow is to be tested, the flanks, belly, and udder of the animal should first be washed and the milker's hands must be thoroughly cleansed. The fore milk from each teat should be discarded, then small sterile bottles may be directly filled, one from each quarter.

When it is desired to examine a mixed milk supply, the vessel containing the milk must be thoroughly shaken, or stirred with a scalded rod. A dipper, which has just previously been scalded and which has not been allowed to touch any unsterilized surface, should be employed to fill the bottles.

Having replaced and secured the linen covers, a label bearing a distinguishing mark should be affixed to each bottle.

All such samples should be immediately packed in an ice-box and sent to the Institute without delay. Under separate cover particulars relating to the samples should be sent on a form supplied with the ice-box.

When examination for the presence of the tubercle bacillus is desired, larger quantities of milk—at least a pint—are necessary.

Suitable sterile bottles and ice-boxes can be supplied on application.

The ordinary routine examination of milk comprises an estimation of the total content of organisms growing at blood-heat, and the presence or absence, in varying quantities of the sample, of *B. welchii* (*B. perfringens* or *B. aerogenes capsulatus*). The first estimation gives indications as to the conditions

under which the milk has been kept since the time of milking, the second is an indication as to the presence or absence of original faecal pollution.

Ice Cream.

Samples of ice-cream are examined in much the same fashion as samples of milk.

A sterile 2 oz. bottle should be filled with a sample of the ice-cream to be examined and immediately transferred to an ice-box and forwarded for examination with the least possible delay.

Disinfectants.

The value of a disinfectant is not to be judged merely by its price per gallon, but in terms of its cost per "Disinfection Unit."

The "Rideal-Walker" method for estimating the carbolic acid co-efficient is the test commonly employed for determining the efficiency of a disinfectant, and the ratio between the figure so obtained and the cost of the material gives the unit cost of the disinfectant.

For the purpose of this test at least a quart of fluid, or a pound of a powdered disinfectant, should be sent, and the sample should be submitted in an air-tight receptacle.

Methods for Preserving Insects, Worms, Etc., for Identification.

Mosquitoes, Biting Flies, etc.

These may be pinned with the fine entomological pins on to the under surface of the cork of a specimen tube, or they may be wrapped loosely in tissue or toilet paper, placed in a box, and the box filled (but not too tightly) with sawdust containing a drop or two of carbolic acid. On no account wrap them in cotton wool.

Mosquito larvae may be preserved by dipping them into hot 70% alcohol containing a few drops of glycerine, or by putting them into water containing 10% of formalin.

Ticks may be sent alive in a well corked tube or bottle, or they may be killed by dropping into boiling water and then transferring to absolute alcohol or strong spirit.

Fleas and Lice should be put directly into absolute alcohol or strong spirit.

Worms may be preserved in 10% formalin or 70% alcohol, but they make better museum specimens if the following directions can be carried out.

Nematodes (Ascaris, Ankylostomes, etc.).

Shake up alive for a minute or two in a test-tube containing normal saline solution. Pour of the saline and quickly add boiling 70% alcohol.

Small Trematodes (Liver flukes and Lung flukes).

Treat as for Nematodes.

Large Trematodes.

First shake up in saline as for small flukes. Add to it a tenth of its volume of pure formalin. Continue shaking till killed. Dilute with an equal quantity of water.

Cestodes (Tape Worms).

Shake *gently* in normal saline for a minute or two. Treat with formalin as in the case of large trematodes.

Worm Ova (in faeces, etc.).

These may be preserved excellently by adding a tenth of its volume of formalin to the specimen and shaking up gently until a more or less uniform emulsion is obtained.

Snakes.—Preserve in 10% formalin or 30% alcohol. Make one or two slits on the ventral surface opening into the body cavity.

Laboratory No. 21.

PART III.

Laboratory Products in Prophylaxis and Treatment.

In addition to carrying out diagnostic procedures the modern medical laboratory plays a considerable part in prophylactic and curative medicine by supplying preparations, mainly of bacterial origin, for use against certain diseases. The function of these products, chief amongst which are Vaccines and Sera, is, broadly speaking, to increase the immunity or powers of resistance of the body against particular organisms.

We shall first briefly consider the general nature, manner of preparation, mode of action, and method of employment of these products, and shall then discuss their use and value in specific diseases.

Bacterial Vaccines.

Nature.

A bacterial vaccine consists essentially of an emulsion of the bodies of the causal organisms of any particular disease. Injection of these bacterial bodies into healthy tissue results in the appearance, in the blood of the individual inoculated, of various "immune" or "anti" bodies—opsonins, agglutinins, bacteriolysins and so forth—which have a specific action against the bacteria in response to which they have been elaborated.

Isolation of Organisms.

The identification of causal organisms is essentially a laboratory procedure, but the co-operation of the practitioner is required in the collection of specimens, and if this be not done with due precautions (as described in Part II.) to obtain the proper material and to prevent contamination, vaccine treatment is scarcely worth while considering.

After organisms have been obtained and isolated in pure culture they may be treated in various ways in order to make up the actual vaccine which is issued for use.

Preparation.

Vaccines are sometimes employed which consist of live bacteria, usually, however, only after the virulence of such bacteria has been diminished by special methods of growth. Similarly, live vaccines which have been "sensitized" (see p. 100) have occasionally been made use of, but as no live bacterial vaccines are in general routine medical use they need not be further considered here.

In practically all the vaccines in everyday use at present the bacteria have been killed. Provided that this sterilization of the bacteria is carried out with certain precautions the dead germs still possess the power, when injected into the body, of bringing about the elaboration within the system of the antibodies which are inimical to the live bacteria (or their products) of the same species.

Organisms for a vaccine, therefore, after being isolated in pure culture and grown in sufficient quantity, are removed from the medium on which they have been grown, and, with suitable precautions to avoid contamination, are suspended in normal saline solution and killed. Sterilization may be effected either by heat or by antiseptics.

Heat was the agent originally employed for killing the bacteria of a vaccine, and it is still used to some extent; exposure to temperatures of from 53°C. to 63°C. for a period of about half-an-hour is usually resorted to. As, however, excessive heating has a markedly deleterious action on many vaccines, the lower the temperature that can be utilized the better, and a common practice is to expose the vaccine to a temperature of only about 53°C. and to complete the

Sterilization.

sterilization by adding, when cold, some antiseptic, such as phenol (0.5%) or tri-cresol (0.3%). Some such preservative is, in any case, practically always added to vaccines before they are finally tubed and issued. In the case of some organisms the application of heat, even of so comparatively low a degree as 53°C., has been shown very definitely to impair both the keeping qualities and the immunizing power of the vaccine, and sterilization at air temperature by means of phenol (in a strength of 0.5% of the saline in which the bacteria are suspended) is much to be preferred in such cases. One standard method may not be the best for all species of bacteria from which vaccines are prepared, but in the majority of cases sterilization by 0.5% phenol or 0.3% tri-cresol appears to be the most satisfactory, producing a vaccine which keeps better, has better immunizing properties, and is less irritating when injected. This method is the one which is most widely employed at the Institute.

Sterility Controls.

Stringent precautions are taken to ensure that every vaccine (or serum) issued is sterile and safe for use. Every container is numbered and initialled, so that in the case of any question arising there is no difficulty as to the fixing of responsibility. The responsibility is in every case a personal one and not merely a vague "institutional" one.

In the case of autogenous vaccines, and of stock vaccines and sera in bulk, *i.e.*—in bottles containing more than one dose, each individual bottle is tested separately for sterility, and we have only one rule, viz., "if there is any doubt, do not pass it."

With vaccines, etc., put up in single doses, it is manifestly impossible to test each ampoule separately, as no material would be left for use. In this

instance the material is tested in bulk before filling into ampoules; if it is passed as sterile, the ampoules are filled with every precaution to avoid contamination, and then 1 out of every 50 or less, taken at random, is re-tested. Should any be found to be unsterile — which practically never happens — the whole batch would be rejected.

Standardization.

Before final tubing or bottling, the vaccine is standardized as to its bacterial content; the number of organisms per c.c., usually stated in millions, is the criterion commonly adopted to indicate the strength of a vaccine. Practically any strength can be prepared, but for convenience in practice the dose, or range of doses, put up is usually such that those amounts which trial and experience have shown to be most suitable can be inoculated in a volume of 1 c.c. or some convenient fraction thereof.

Sensitized Vaccines.

Sensitized vaccines are vaccines which have been treated with their specific anti-sera. After such treatment the organisms are usually killed in the ordinary way, although living sensitized bacteria have occasionally been employed in treatment. Such a method cannot be said to be entirely without danger, however, and cannot at present be recommended as a routine measure. Sensitized vaccines cause less reaction than ordinary vaccines do and, although their preparation involves a considerably more elaborate process, they have been employed with considerable success in treating Typhoid, and Bacillary Dysentery, two conditions in which the ordinary vaccine may cause an undesirable degree of reaction. They have also been employed in other conditions, but without the same outstanding advantage over the ordinary vaccines.

Keeping of Vaccines.

With the lapse of time vaccines tend gradually to lose their immunizing properties. Vaccines sterilized chemically preserve them longer than do those sterilized by heat, and vaccines kept in cold storage preserve them longer than those kept at ordinary air temperatures. The loss of immunizing properties is a gradual one, and the rate of loss varies with different organisms, and may also depend on a number of other factors.

We do not ourselves stock or issue any vaccine over six months old, and we do not *recommend* the use of any vaccine of an older date than that, more especially if it has not been kept in cold storage. It might be efficient, but it is just as likely to be useless. It is not advisable therefore to order larger amounts of vaccine than can be used up quickly, and all samples should be kept in as cool a place as possible and away from direct bright light.

Mode of Action.

The mode of action of a bacterial vaccine, when used in treatment, is still by no means completely understood; the dosage, frequency of administration, etc., are, therefore, still in some degree empirical. When the method was first introduced by Wright it was hoped that it would be a strictly scientific one and capable of being exactly controlled by observations of the opsonic index. The method has been generally adopted, but the opsonic index control has been largely dropped, and vaccines are now being used in a multiplicity of conditions in which the only control is that afforded by clinical results. Even these are sometimes scarcely considered, the vaccine being blindly injected once a week according to some graduated system of dosage supplied ready-made by the manufacturers. We do not wish to imply that vaccines should be used in season and

out of it for every conceivable complaint, but such a system of employing them as has just been referred to is an abuse of a valuable method and one which often leads to the condemnation of all vaccines as useless—whereas it may well have been the method of using them which was at fault.

So far as the prophylactic use of vaccines is concerned the amount and frequency of dosage may be said to be fairly well standardized for the majority of conditions against which they are employed. The degree and duration of the immunity conferred is less precisely known and doubtless varies somewhat in different individuals, but approximations to the truth can be stated. The immunity conferred is, in the main, a specific one, *i.e.*, the anti-bodies formed give protection only against the organisms or strains of organisms of which the vaccine is composed.

In the therapeutic use of vaccines our ideas as regards specificity have in recent years received several rude shocks. By intravenous injections in, for instance, Typhoid Fever, a marked reaction has been produced, with, in quite a big proportion of cases, a cure by crisis; yet in such cases it seems to matter little whether the inoculum consists of typhoid vaccine or of a vaccine of some other organism, or even simply of a protein of foreign origin, *i.e.*—derived from some animal species other than the human one. This non-specific character of the reaction has given rise to the expression, "treatment by protein shock," and it is quite possible that in the future the method may play a considerable rôle but at present it scarcely comes within the purview of routine practice.

All that can be safely affirmed at present is that the action of vaccines when given intravenously differs considerably from that when they are exhibited subcutaneously; and that the non-specific element plays a greater part in curative than in prophylactic work.

Other remedial measures in ordinary medical and surgical employment should never be neglected, particularly the clearing out of any pockets of pus which may cause re-infection, and the elimination of "toxins" by diaphoretics, diuretics, and enemata.

Concomitant Treatment.

Since the object of a vaccine is often to determine the flow to a particular focus of antibacterial bodies and efficient phagocytic cells, any measure which will promote this is to be encouraged, *e.g.*— the scraping of sinuses, relief of tension by incision and drainage, hyperaemic congestion, irrigation with hypertonic saline, etc.

Opinion as to the best doses of vaccines is still in a comparatively fluid state. Originally they were given in minimal doses, with the idea of gradually working up the opsonic index; now, the tendency is towards large doses and quick results. The increase of dosage is most striking in certain acute infections; where patients used to be given 5 or 10 millions with some fear and trembling they are now being given doses running into thousands of millions. This change of practice has undoubtedly been for the better in some instances.

Dosage.

There is considerable individual variation in the response to the inoculation of a vaccine. If, for instance, a large number of persons be inoculated with a like dose of the same batch of a prophylactic vaccine, a divergent series of reactions will be noted amongst them, from *nil* up to reactions sufficiently severe perhaps to lay the individuals up for a day or so. Similarly in therapeutics, the dose which is beneficial in one case may be uselessly small or harmfully large in another.

In practice, therefore, every case treated with a vaccine should be regarded as an individual experiment, and the amount and frequency of dosage should be regulated by the observed reaction to the preceding dose. From accumulated experience, we are able to state the dose or range of doses which has been found most generally useful in any particular class of cases; but the man who uses vaccines in a purely mechanical manner and expects to get consistent results by injecting a fixed number of doses at fixed intervals according to some cut-and-dried plan, is going to have many disappointments and probably more failures than successes. It would be delightfully simple if such a "fool-proof" method of giving vaccines could be devised, but so long as individual variations amongst men and bacteria continue to occur there is little prospect of its coming about.

To give a vaccine a fair trial and to get the maximum number of good results from vaccine therapy it is essential, therefore, that patients should be seen, at least once, on the day after inoculation, and that further treatment should be based on the observed reaction following on the injection.

It is a universal rule in vaccine work that unless a vaccine produces some reaction, it is either useless or the dose is inadequate.

The reaction following an inoculation may be *local, focal,* or *general.*

Reaction Guide.

The local reaction at the site of inoculation is not much guide as to the suitability or otherwise of the dose, although with a dose which is not excessive, the local reaction is, as a rule, nothing more than a little redness with a swelling which is just slightly tender. The focal and general reactions are of much more importance.

An adequate dose is followed by such *general symptoms* as headache, sleepiness, rise of temperature of not more than 1.5°F., increase of pulse-rate by about 10 or 15 beats per minute, and slight feeling of malaise. These symptoms may last for about 12 to 18 hours and then pass off. The *focal reaction* is manifested by an increase of the physical signs at the site of the lesion and by a definite though slight exacerbation of the symptoms. Any one or more of these effects constitutes "a reaction." Within 24 to 36 hours the malaise should have passed off, and have been succeeded by a feeling of increased well-being, the temperature should have dropped and any fluctuations characteristic of the illness should have been definitely diminished, the pulse should have slowed, and the local lesions or symptoms should show some definite improvement. So long as any given dose produces these effects it should not be increased, but may be repeated after a suitable interval, usually about 4 or 5 days; when it ceases to produce a definite reaction, the size of the next dose may be increased by from 50 to 100%.

An excessive dose is one that produces too violent a reaction, such as a rise of temperature of more than 1.5°F., an increase of pulse-rate of 20 or over, vomiting, severe constitutional disturbance, and intense aggravation of the focal symptoms—all of which are not shortly followed by subsidence and improvement. In these circumstances another dose should not be given for at least 7 or 10 days, and should then be much smaller, a half or even less. If it is then found to be adequate the advice given in the preceding paragraph should be followed.

An insufficient dose produces no appreciable effect, and subsequent doses may be doubled on each occasion until a definite reaction is obtained.

Infants and children should be given smaller doses than adults—from one-tenth to three-quarters of the adult dose, according to body-weight. One-twentieth of the dose for an adult multiplied by the age of the patient in years is a convenient approximation. Patients of very advanced years or in a very debilitated condition should also receive reduced doses at the commencement of treatment.

General Considerations.

Before concluding that a case is not amenable to vaccine treatment at least four or five doses should have been given, when, if no improvement is observed, the vaccine should be discarded and a new one prepared; for this purpose a fresh sample of material should be supplied. The second attempt sometimes succeeds admirably, especially in infections of the respiratory and urinary tracts.

The inoculation is best carried out in the late afternoon or early evening, and the patient should stay in bed, or at least, remain quietly in the house, until the following morning, when he should be seen by the practitioner, in order that the reaction to the inoculation may be properly gauged. This plan has also further advantages in that (1) The possibly disagreeable reaction occurs during the night, and (2) The patient passes through the "negative phase" under the most favourable conditions. The negative phase—*i.e.*, the period after an inoculation during which the patient's resistance to his infecting organisms is lowered and he is more liable to attack—was at one time the great bogey of vaccine therapy. It is now generally regarded as of little practical importance and can be disregarded except in cases of catarrhal conditions of the respiratory tract; in these it is of significance, and it is important that for the twelve hours or so following on an inoculation the patient should not be exposed to chills or possible extraneous sources of infection.

Administration of the Vaccine.

Vaccines have been given both orally and rectally, but, although a certain amount of immune-body formation may follow, it is usually small in amount and of uncertain occurrence. The employment of these routes can only be advocated, therefore, in the rare instances in which there are serious objections to the use of a syringe. Intramuscular injections have also been shown not to be so productive of antibody formation as subcutaneous inoculations, so that the subcutaneous route may be considered the only practical one for the routine administration of vaccines.

Site for inoculation.—The usual site selected is one of the following:—(1) the flank, (2) from 1½ to 2 inches below the centre of the clavicle, (3) the outer surface of the upper arm.

Preparation of the skin.—Disinfect the skin, at the area selected, with Tincture of Iodine.

Withdrawing the vaccine.—(a) *From an ampoule.*—Sterilize a hypodermic syringe by boiling, and affix the needle. Shake the ampoule vigorously for a few seconds, then give it a sharp downwards jerk in order to dislodge any fluid remaining in the stem. Cover the stem with a pad of cotton-wool or lint and grasp it between the thumbs and forefingers with the thumb-nails close to one another; a slight bending strain will now cause the stem to snap. Any fragments of glass that may splinter off will remain in the pad of wool or lint. The broken ampoule should now be inverted over the needle and the desired volume of vaccine drawn into the barrel of the syringe. (b) *From a rubber-capped bottle*—Similarly sterilize a syringe and affix the needle. Shake up the contents of the bottle. Place a drop of Lysol, Tincture of Iodine, or other strong disinfectant on the cap. Thrust the needle through the disinfectant and cap into the vaccine, reverse the bottle if necessary, and draw up the volume

required. To close the opening wipe off the disinfectant and seal the puncture by lightly touching the wax coating with a burning match or hot wire.

Injecting the vaccine.—Grasp a fold of skin between the thumb and forefinger of one hand; holding the syringe in the other, boldly plunge the needle through the skin, gradually inject the fluid, then quickly withdraw the needle.

Stains of Iodine may be removed from the skin, if desired, by washing with solution of Hyposulphite of Soda (photographers' "Hypo.").

Sera.

In serum therapy the patient is not actively immunized as in the case of vaccine therapy; in the latter the injection of bacteria brings about the direct formation of anti-bodies within his system. The immunization in this instance is a passive process; the antibodies being injected "ready-made," having been already elaborated in an animal in response to inoculation of it with the particular organism concerned.

Nature.

The action of the immune bodies in a serum may be, for the most part, directly on the invading bacteria, *i.e.*—a bacteriolytic or bactericidal action, as in the case of anti-streptococcal or anti-meningococcal serum; or it may be mainly that of neutralizing the products of the bacteria, *i.e.*—an antitoxic action, of which class diphtheria and tetanus antitoxins are good examples.

Passive immunization by means of sera may, like active immunization by vaccines, be for two distinct purposes—for prophylaxis, or for treatment. The same serum may serve both ends, but only two, so far, have established their place as thoroughly efficient for prophylactic use, viz., tetanus and diphtheria antitoxins. The former is, indeed, of far more value as a prophylactic than as a curative

agent after definite symptoms have made their appearance. Naturally, however, seeing that the immune bodies are introduced from without and that no stimulus is given to auto-production, the immunity given by such means cannot be expected to be of long duration, and such passive immunization for prophylactic purposes is only indicated in the face of imminent danger of infection.

For curative purposes, re-enforcements, in the shape of specific antibodies, may be of great assistance in acute diseases where the body-cells are hard pressed in their attempts to repel the invading bacteria or to neutralize their poisons. Generally speaking, the more acute the infection the greater is the indication for the exhibition of an antiserum in any disease in which one is available.

Preparation of Sera.

The number of diseases against which it has been found feasible to produce efficient sera is still very small, although there is every hope that the number will be increased in the future. Their manufacture is a highly technical process which it would be out of place to describe in any detail here, more particularly as the only specific antiserum which is prepared at the Institute at the present time is Anti-Dysenteric serum. The preparation of others which are of known value is, however, an extension of our activities which we hope will come about in the near future. Meanwhile, we have to content ourselves with stocking sera manufactured by others, as a convenience to our clients.

Keeping of Sera.

Sera are, even much more than vaccines, sensitive to heat. At the air-temperatures commonly met with in South Africa they will quickly deteriorate and lose their immunizing properties. It is very inadvisable therefore, unless cold storage is available, to attempt to keep any stock of serum

on hand for emergencies. Rather obtain it when required by urgent order from some depôt where it is kept under suitable conditions.

Dangers of Serum Therapy.

Provided that the sera are properly made the risks run in using them are very small; that which is apt to weigh most on one's mind is the risk of *anaphylactic shock*. It was noticed, a number of years ago, that after the introduction of a foreign protein, such as horse serum, a second injection of the same protein was sometimes rapidly followed by symptoms of respiratory embarrassment, convulsions, and even death. To this phenomenon the term *anaphylaxis*, indicating the opposite of prophylaxis, was applied. The first injection apparently sensitizes the body-cells to the particular foreign protein, but it requires an incubation period of *about ten days* before the sensitiveness develops. The risk of an anaphylactic accident occurring is, however, so remote that it need only very rarely interfere with the use of serum when occasion demands it. The chances, small as they are, may be minimized by taking the following considerations into account. (1) Whether the patient is naturally sensitive to horse-protein. It is of importance to know this because in some of the cases in which anaphylactic shock has occurred it has been afterwards ascertained that they were thus sensitive—they could not, for instance, go near horses, without developing such symptoms as sneezing, asthma, or urticaria. The horse is the animal used for the manufacture of most of the sera on the market, and it is solely because the serum is of non-human origin that it may give rise to trouble; the antibodies which it may contain are quite harmless. If there is any doubt about the matter it may be settled experimentally in about twenty minutes. Scarify the arm as if for vaccination with calf-lymph, and rub into the scarifications a little diphtheria anti-

toxin or normal horse serum—it matters not which; if the patient be naturally sensitive an urticaria-like rash will develop around the scarifications in about a quarter of an hour. In such a case the precaution detailed in the next paragraph must be adopted. (2) Whether the patient has ever previously had an injection of horse serum, antitoxic or normal. Such an event may have happened, for instance, to a patient who has at some time or other been treated for Diphtheria by means of antitoxin, or who has received a dose of antitoxin as a prophylactic against that disease. In such an instance, it is sound practice, a couple of hours before administering the therapeutic dose (which should not be withheld simply on account of the remote risk of anaphylaxis) to inject subcutaneously about 0.5 c.c. of antitoxin or of normal horse serum. This small amount is of itself very unlikely to produce serious anaphylactic symptoms, but it tends to bring about an anti-anaphylactic state which makes the giving of the large amount a safe procedure. Should *any* symptoms follow the administration of the small anti-anaphylactic dose caution would naturally have to be exercised in giving the larger.

It is a sound rule *never to give a second injection of a serum intravenously.* Although it is good practice to give the first by that route, for the sake of rapidity of action, it is advisable to give any subsequent doses subcutaneously or intramuscularly. Should any anaphylactic symptoms then ensue they are not likely to be so explosive or serious as if the serum had been introduced directly into the circulation by way of a vein.

In asthmatic subjects serum injections may give rise to some anxiety through respiratory embarassment and in such cases serum should not, as a rule, be given intravenously.

Apart from these conditions, untoward effects have followed serum injections practically only in cases

of the *status lymphaticus,* and for such no remedy has been suggested.

"Serum Sickness," comprising rashes, joint-pains, oedema, etc., may develop upon an injection of serum after an interval of 8 to 10 days or so. It is an anaphylactic phenomenon due to the action of unabsorbed protein on the sensitized tissues; it need never give rise to any serious anxiety.

Dosage and Route of Administration.

The amount of serum given and the route by which it is administered are very important factors in deciding the success or failure of serum treatment. These will depend very much upon the individual case, and the question will be further referred to under the particular diseases in which serum is employed. Generally speaking, however, the object aimed at, especially in curative immunization, is rapidity and sufficiency of action, and if there is any doubt as to the amount which should be given err on the side of a large dose rather than on that of a small one; an excess of antibodies is very unlikely to do any harm, whereas a deficiency of them may easily be fatal. As regards the method of administration, select that which promises to get the serum to the desired spot most quickly.

According to circumstances, serum may be given subcutaneously, intramuscularly, intravenously, or subdurally. The *subcutaneous* route, which is the slowest so far as absorption is concerned, is suitable for prophylactic injections against Diphtheria or Tetanus, for second or subsequent injections in practically any case, and for patients in whom an anaphylactic reaction is feared for any reason. It may also be employed for curative administration of diphtheria antitoxin in mild cases. The injections are usually made in some area with loose subcutaneous tissue, such as the abdominal wall, or the back in the neighbourhood of the lower angle of the

scapula. The syringe used should be of from 5 to 20 c.c. capacity and preferably of glass. Sterilization by boiling is, of course, necessary. The needle should be of medium bore, rather larger than that employed in ordinary hypodermic work; it is a good plan to have it connected to the syringe by a short length of rubber tubing so as to avoid possible accidents through the patient struggling. Large volumes of serum should never be injected at one spot on account of the delay in absorption, and also for fear of sloughing. The more concentrated a serum is the more suitable it is for use, since the necessary antibodies will be contained in a smaller volume.

An injection by the *intramuscular* route is quicker of absorption than by the subcutaneous, and may be employed in any case where speedier action is desired, and where, for any reason, intravenous injection is not suitable. The technique is the same as for subcutaneous inoculations, except that the needle is plunged right into the depth of muscular tissue, the gluteal muscles being those usually selected.

Intravenous administration of serum is the best method to employ in the great majority of cases where a rapid action is desired, but it is important to remember the possible risks in patients who have received serum on some previous occasion. Where relatively small volumes of serum are being given, a serum syringe may be employed; quantities of 20 c.c.s and over are usually more easily administered by some simple gravity apparatus, such, for instance, as is employed for the administration of salvarsan. The technique is very simple. Having filled the sterilized syringe or the sterilized cylinder of the gravity apparatus, and seen that all air-bubbles have been expelled, put a tourniquet round the upper arm—a length of soft rubber tubing fixed by looping under itself serves admirably as it is quickly and easily released; select a prominent vein about the

elbow, disinfect the skin over it with iodine, steady the vein between the left forefinger and thumb and with the right hand introduce the needle. Release the tourniquet and slowly inject the serum, or allow it to flow in as the case may be. The puncture in the skin may be sealed with cotton-wool and collodion or with a pad of gauze and a bandage.

Subdural inoculations are employed in the treatment of Tetanus and of Cerebro-spinal Meningitis. The procedure is by no means always a simple one. Suitable needles such as are employed for lumbar puncture (see p. 73) are required, and a syringe or gravity apparatus which can be attached to them. A general anaesthetic is frequently necessary, and as there is always a danger of collapse the patient must be kept in bed in the lateral position, and atropine and caffeine should be at hand in case of need. Having got ready all the apparatus, and the serum having been warmed up to body temperature by immersion of its container in hot water, perform a lumbar puncture as directed on p. 73. The question now to be decided is how much fluid to drain off and how much serum to inject. The rule-of-thumb method advises that from 15 to 20 c.c.s should be drawn off and an equal amount of serum injected. This may suit some cases, but with thick exudates much less fluid than 15 to 20 c.c.s may be obtained and to inject that amount in such cases may be highly dangerous from the sudden increase of pressure. On the other hand it may be just as dangerous, in cases where the flow is free, to allow it to continue until a flow of a drop every three or four seconds is reached and then to inject the serum. Control by simultaneous observation of the blood-pressure has been advocated as giving the safest guide as to the amounts which may be withdrawn and injected. The blood-pressure usually falls when cerebro-spinal fluid is withdrawn; after a drop of about 10 mm. of mercury (5 in a child) it is advis-

able to stop. If, as sometimes occurs, there is no fall, it is safe to drain until the normal rate of flow, which is a drop about every three seconds, is reached. With the injection of serum the blood-pressure usually falls still farther, the fall being generally proportional to the amount injected and the rate at which it is injected. It is not usually advisable to go beyond a total fall in blood-pressure of 20 mm. of mercury. The temptation to inject the serum quickly and "have it over" must be resisted; it should be injected very slowly, taking not less than ten minutes to inject 15 c.c.s of serum. Such regulation of flow is naturally more easily done with a gravity apparatus than with a syringe.

Other Biological Laboratory Products.

NORMAL SERUM, usually that of the horse, although the species from which it is obtained does not seem to matter in most instances, has been employed with considerable success in a number of diverse conditions. Among these conditions mention may be made of such blood diseases as Haemophilia, Purpura haemorrhagica, Melaena neonatorum, and Simple Anaemia, and of Internal Haemorrhage (from the bowel as in Typhoid, haematemesis from Cirrhosis of the Liver, or haemoptysis from the lungs) Gastric Ulcer, and skin diseases like Urticaria, Pruritus, and Pemphigus. It may be given subcutaneously, or intramuscularly in acute cases when a more rapid action is desired, otherwise it can be taken by the mouth. It has also been used as a local application or dressing to suppurating wounds or ulcers. The latest application of normal serum therapy is in the treatment of Anthrax, large intra venous injections of ox serum having been reported to yield as good results as specific anti-anthrax serum. Ox or goat serum may be employed in

place of normal horse serum in the case of patients who have previously received serum of equine origin and with whom there may be a risk of anaphylaxis.

BULGARIAN SOURED MILK.—This preparation, first recommended to the world at large by Metchnikoff, contains special lactic-acid-producing bacilli, and the idea underlying its use is that these bacilli may supplant other harmfully fermentative organisms in the bowel. It has been used with a considerable degree of success in various gastric and intestinal disorders, and in poorly nourished infants and children. Even should there be no special pathological feature to combat, milk which has been soured by means of the *Bacillus bulgaricus* is enjoyed by many as a pleasant and wholesome article of diet.

Preparation of the Bulgarian Milk.

To prepare Bulgarian Milk with a culture of the *B. bulgaricus* boil a pint, or more, of milk for 5 minutes, then pour it into a clean, previously scalded, jar or bottle. Cover the vessel with a loose cover to protect the milk from flies and dust, and cool it down to about body temperature (98°F.). Now add the culture, which is obtainable from the Institute, and gently stir the mixture with a scalded spoon.

Incubate the mixture by standing the covered vessel in a *warm* place—such, for example, as a cupboard over the kitchen range or a sheltered corner near the stove—for from 12 to 18 hours. When the milk has become thick it is ready for use; if clotting has not taken place within the period named, the vessel should remain undisturbed for a further 6 or 8 hours.

Before the soured milk is consumed add a tablespoonful of it to a further quantity of milk which has been prepared as before. By this means a *continual supply* of the soured milk may be obtained. If the milk does not thicken within the specified

time, a little of it may be employed as a "starter" for a further quantity of milk, which will probably then clot satisfactorily.

Should the brew at any time become bitter, it would be advisable to obtain a new culture.

If the milk, when made, be kept in an ice-box it will preserve its consistence for many days. Iced Bulgarian milk thus prepared is a very wholesome article of diet at all times, and is very refreshing in hot weather.

Note.—Failure of the milk to clot may be due to (1) the culture having been killed in consequence of it having been added to the milk whilst the latter was too hot; (2) the place where the milk is placed to incubate being either too cold or too hot.

SMALL-POX VACCINE.—The organism which causes Small-pox is still unknown, and there is still no completely satisfactory explanation of the relationship between Small-pox, Cow-pox, and Vaccinia. Some still maintain that Small-pox is a separate entity; the "monistic" theory, however, obtains most support, viz., that the virus is one and the same in all these conditions, but that it is so changed by its passage through a lower animal that it loses forever its power of producing Small-pox, although it remains so closely related that the antibodies it produces give protection against Small-pox. Certain it is, whatever the nature of the virus or viruses may be, that Small-pox and Vaccinia are protective against one another for longer or shorter periods.

The general applicability of the last statement is, however, considerably modified by circumstances of race and environment. In warm climates, and amongst dark-skinned races in particular, although vaccination will reduce the incidence of Small-pox in a previously unvaccinated tribe exposed to that disease, yet the degree of immunity conferred in such

circumstances is usually less, and its period of duration shorter, than is generally the case in the white race in temperate climates. It is no very uncommon thing, for instance, to see vaccination taking well in an African Native with well marked pocks; and, unfortunately, the converse also hold good, that Small-pox may occur in a Native who has been fairly recently and efficiently vaccinated. Possibly this has something to do with the well known technical difficulty of producing a potent vaccine lymph in a warm climate—a difficulty which, however, we need not expatiate on further here.

Presuming that an efficient calf lymph vaccine is obtainable, there are still a few points concerned with its use which we consider it advisable to draw attention to—

Storage.

(1) Lymph is extremely sensitive to heat, and at temperatures such as are of everyday occurrence in South Africa will rapidly deteriorate and lose its power of "taking," or, even if it still takes, of giving anything like efficient protection. It will keep more or less indefinitely if kept thoroughly chilled, but not otherwise. It is recommended, therefore, always to obtain lymph as fresh as possible and not to attempt to keep it for long periods unless there is proper cold storage available.

Vaccinating.

(2) Lack of attention to two little details, especially when large numbers have to be vaccinated at one time, leads to a good deal of unsatisfactory vaccination. These are (*a*) Leaving antiseptics on the arm. The arm should certainly be cleansed before scarification — and this not always an easy matter with a Native skin—but any antiseptic used should be removed with clean water before the lymph is actually applied. (*b*) Vaccinating in bright sunlight. The African sun will

quickly kill out the Vaccinia virus; patients should, therefore, be vaccinated indoors or in the shade, and their arms should not be exposed to direct sunlight until the lymph has thoroughly dried in.

Use of Vaccines, Etc., in Individual Diseases.

Preliminary Note.

At the risk of producing weariness we would reiterate the statement that the doses of vaccines, etc., mentioned on this and the following pages are only intended as general guides and should not be adhered to blindly. Consistently satisfactory results can only be expected if each case is treated on its own merits according to the principles enunciated on p. 103 *et seq.*

Only the more important conditions in which treatment with vaccines, etc., is commonly employed can be dealt with here. Reference to any standard work on the subject, such as Allen's "Vaccine Therapy," will show many more diseases in which they may be employed, and will supply in much greater detail the particulars regarding the use of vaccines in practice.

ABSCESSES, SEPTIC WOUNDS, ETC.—In these, vaccine treatment is not infrequently a useful adjuvant, especially in chronic cases, although it will naturally but seldom be the only measure adopted. The common pyogenic cocci are the organisms usually found to be present, and a stock "Pyogenic Vaccine" containing a number of strains of Staphylococci and Streptococci may be tried initially. If found satisfactory it may be continued, but if there is no response it will be well to have the pus or discharge investigated with a view to ascertaining definitely the causal agent and, if considered advisable, preparing an autogenous vaccine. In acute cases doses of 25 millions at intervals of 3 or 4 days may be sufficient; in more chronic cases an

initial dose of 100 millions will probably be found more satisfactory, with subsequent inoculations when there is any sign of retrogression, or, say, from 5 to 10 days later. After recovery it may be advisable to give at intervals of a month or two a few really large doses to prevent recurrence, *e.g.*, 1,000 or 2,000 millions; but naturally much will depend on the individual circumstances of the case whether this will be considered necessary or not.

In severe cases, shown definitely to be streptococcal in origin, anti-streptococcal serum may be employed in a fashion similar to that recommended for Cellulitis (See p. 123).

ACNE.—A stock "Acne compound Vaccine" containing both Staphylococci and the Acne bacillus (numerous strains of each, and in suitable proportions) is often just as satisfactory as an autogenous preparation, and by some writers is, indeed, preferred. It is only exceptionally, when a stock vaccine has been found not to work, that an autogenous one is really necessary in this condition. A dose of 100 millions will practically never be found too large for an initial dose, and subsequent doses may be given at intervals of from 5 to 7 days or just when any retrogression of the condition is observed to occur. It is necessary, as a rule, to push the treatment till doses of 1,000 or 2,000 millions are being given, and even then it is a lengthy business. A marked degree of improvement is usually comparatively easy to obtain, but an absolute cure may be difficult with one series of injections. It often seems advisable, once this stage has been reached, to give a large (four figure) injection at longer intervals, say, about three weeks, than to continue them at about weekly periods.

Note :—

B. acnes is a slow grower and an autogenous vaccine containing this organism takes 2—3 weeks to prepare, as against the more usual 4—6 days.

ANTHRAX.—The vaccine first introduced by Pasteur for the prevention of Anthrax in cattle, sheep, etc., and still used for these purposes with conspicuous success in veterinary practice, consists of a live culture of *B. anthracis,* but one which, however, has had its virulence attenuated by cultivation in a special manner. The results obtained in animals with this vaccine are highly satisfactory; nevertheless a few fatalities do attend its use, so it is obviously unsuited for employment in the human subject. Various sera have been employed in the treatment of Anthrax, the most valuable being that made according to Sclavo's method. Statistical evidence certainly appears to show a considerable diminution of the case-mortality when using it, but so many cases of ordinary external Anthrax (provided that the local lesion is not situated on the head) recover under surgical treatment alone, that it is a little difficult to evaluate the evidence properly. More recently salvarsan has been used with apparently equally good results, but again the same criticism applies. A vaccine of killed bacilli has recently been tried by us, but we have not yet sufficient evidence to appraise its value, although the results given in one bad case were very encouraging. The latest remedy is normal serum, by the use of which highly satisfactory results have been reported in a fairly large series of cases. The presumption is that Sclavo's serum may also owe its efficacy mainly to its foreign protein and only in small part to specific antibodies. Any foreign protein may answer, but normal heated ox serum has the advantage over horse serum of not causing serum sickness. In the present somewhat unsettled position of this question we would advise that when dealing with an actual case Sclavo's serum, if it is available, should be given, but that bigger doses than usually recommended be employed. Ten to twenty c.c.s has been the amount usually given, but

there is little reason to doubt that even in a mild case from 20 to 50 c.c.s (intramuscularly or intravenously) would be more suitable—in addition, of course, to excision of the pustule or other surgical measures. In severe cases, spreading or showing bacilli in the blood on culture, 100 c.c.s given intravenously and repeated daily if need be, would be advisable. If Sclavo's serum is not obtainable normal ox serum may be used in a similar manner and in similar amounts. In a case of internal Anthrax all that could be done would be to try either salvarsan or large doses of serum intravenously.

ASTHMA.—True Bronchial Asthma is, in a large proportion of cases, very amenable to treatment by an autogenous vaccine prepared from the patient's sputum. The most important preliminary consideration is the proper collection of true bronchial secretion coughed up during an attack. In order to obtain the actual causative organisms as free as possible from any mouth contamination the instructions given on p. 76 should be strictly carried out. The organisms found may vary considerably in different cases, but streptococci seem very frequently to play an important rôle in the etiology of the disorder. The dosage will naturally vary with the class of organism found and also with the patient, but, as a general rule, very large doses are not required. A trial of a few doses will, in most instances, soon show whether it is worth while persevering with vaccine treatment or not. If the vaccine is found at first to precipitate or aggravate an attack, improvement can practically always be promised. Doses should not as a rule be given oftener than once in ten days, or seven days as a minimum, and they should never be increased beyond what is required to produce a very slight reaction. Sometimes an absolute cure can be obtained, but even if

this desideratum be not attained there is frequently such a degree of relief given that a trial is at least always worth considering. Even when the limit of maximum benefit appears to have been reached it is advisable for the patient to have an occasional dose, or perhaps a series of two or three, in order to prevent relapse. No other appropriate therapeutic measure need, of course, be neglected while vaccines are in use.

BOILS.—The manner in which boils will clear up under vaccine treatment is one of the delights of practice, and is as satisfactory to the patient as to the physician. An autogenous vaccine is but rarely required, a stock staphylococcal preparation usually serving just as well. 100 millions is an appropriate initial dose, and subsequent injections may be given at intervals of from 5 to 7 days. Some slight focal and constitutional disturbances should be aimed at with the first few doses. It will probably be necessary to work up to at least 1,000 millions to obtain a lasting immunity, and it is advisable, as a rule, to give one or two full doses at intervals of about a month after the condition has completely cleared up in order to ensure the permanence of the cure.

CELLULITIS (Streptococcal).—In Cellulitis definitely shown to be of streptococcal origin anti-streptococcal serum has not infrequently been employed with happy results. In other streptococcal infections, such as Erysipelas, Puerperal Septicaemia, and Endocarditis, the results have not been very encouraging. Since there is, in most streptococcal infections in which serum may be thought of, a systemic infection, the uselessness of 10 c.c.s of serum subcutaneously is almost self-evident. It can do no harm in almost any amount, and may do good if used boldly. If using it at all give 100 c.c. intravenously, and repeat with similar

or somewhat smaller amounts in twelve or twenty-four hours — intramuscularly or intravenously according to the severity of the case. There is unfortunately no satisfactory method of standardizing this serum, and as it quickly loses its protective value unless stored under ideal conditions (and even then it goes off gradually) there is little doubt but that much of the stuff on the market is utterly worthless.

CORYZA, COLDS, AND OTHER RESPIRATORY CATARRHAL CONDITIONS.—Practically all persons subject to recurrent catarrhal affections of some part or other of the respiratory tract are suitable subjects for vaccine treatment. Naturally some will respond better to treatment than others, and a good deal depends also on the portion of the respiratory tract most affected. The outlook, for instance, is not so promising when parts with a comparatively poor blood supply, like the accessory sinuses, are involved, as it is in pure nasal or bronchial cases; in the latter the treatment may make all the difference between a comfortable and an intolerable life for the patient.

In some cases the recurrent affections seem always to be due to one particular organism, and in such instances an autogenous vaccine prepared from the patient's own strain is clearly indicated, in others there appears to be a lessened resistance to all organisms liable to set up respiratory catarrhal conditions, and in these treatment by a stock vaccine containing a large selection of appropriate bacteria will probably be the more effective. The difficulty comes in, of course, in knowing to which class any particular case may belong, unless the patient is prepared in the interests of science to await the results of repeated examinations during an extended series of attacks, and few are so self-sacrificing. But certain it is that some cases do better on an

autogenous vaccine than on a stock compound one, and *vice versa*, and, if the truth were known, the explanation is probably that offered above. We have some clients who swear by a stock preparation for every case, whilst others require an autogenous vaccine for each one. Our advice to any practitioner who has no such decided opinions is, first try a stock compound vaccine, then, if that is not found satisfactory, try an autogenous one. In all cases, of course, the general principles governing dosage, as described on pp. 103 *et seq.* should be strictly adhered to, and particularly that inoculation should, if possible, be late in the day. It may be said that there is not often a likelihood of producing a permanent immunity against infection in such conditions, but a very marked relief from infections can frequently be obtained. There is no point, therefore, in trying to push dosage too far or for too long. A stock Coryza Compound Vaccine is prepared at the Institute containing a large number of strains (from different sources) of the organisms commonly found in respiratory catarrhal conditions — Streptococcus, Micrococcus catarrhalis, Pneumococcus, Pneumobacillus, Bacillus septus, Bacillus influenzae, etc. New strains are frequently added to this preparation and it is varied in its proportions from time to time as the prevailing types of infecting organisms seem to be changing; it is put up in five graduated strengths (Nos. 1 to 5) the relative strengths of which are proportionate to the numbering; No. 5, for instance, has five times the bacterial content of No. 1. Speaking generally of treatment with this, from five to ten doses will usually be sufficient for a series, about five days being a suitable interval between doses in most cases. Commence with a No. 1 and, judging by the reaction produced, gradually work up to the stronger doses, never, however, going on to a stronger dose so long as an efficient

reaction is produced by a weaker one. If no benefit appears to result, a bacteriological examination with a view to a possible autogenous vaccine is probably indicated. If improvement does follow, it will probably reach its maximum after from five to ten doses; but practically always it will be found a sound plan to re-inforce the patient's immunity at occasional intervals by one or two injections of the strongest dose to which he has been worked up to before.

CYSTITIS.—This is usually due to an organism of the *B. coli* group, either alone or, sometimes, in combination with other organisms. For this and allied conditions due to coliform bacilli a stock vaccine containing a variety of strains of *B. coli* and allied organisms may be employed, but an autogenous vaccine is much to be preferred. Treatment with such is usually highly satisfactory. Initial doses may be from 25 to 100 millions, and appropriate intervals between doses may be from 3 to 7 days. No suitable surgical measures should, of course, be left unattended to, but should the infection be one complicating Tuberculosis the satisfactory results which usually attend the treatment of simple cases need hardly be expected.

DIPHTHERIA.—*Use of Antitoxin in* (*a*) *Prophylaxis.*—This is probably advisable for close contacts in a family or small community. A subcutaneous dose of 500 units for an infant, or 1,000 to 1,500 units for an older child or adult, is probably quite sufficient. The immunity afforded lasts for from two to four weeks. The only drawback to the practice is the possible risk of anaphylaxis if the patient should develop Diphtheria at a later date and require antitoxin treatment. (See p. 111).

(*b*) *Treatment.*—While antitoxin treatment by no means constitutes the whole treatment of Diphtheria its absence might be likened to a performance of

Hamlet without the Prince, for the evidence of the enormous lowering of the case-mortality in Diphtheria, as the result of antitoxin treatment, is overwhelming. Diphtheria antitoxin is now accurately standardized, the unit being that amount which will just neutralize 100 times the minimum lethal dose of toxin for a 250-gramme guinea-pig. It is possible, therefore, accurately to regulate the dosage by the number of antitoxic units and not merely to approximate it by altering the volume of a serum of unknown strength. The more concentrated the serum the better, since the same number of units is then contained in a smaller volume of fluid. How many units of antitoxin should be given? The answer to this question will naturally vary with circumstances, but, generally speaking, a smaller dose administered early is better than a larger dose administered late. It is of great advantage that the serum should be quickly absorbed, particularly in severe cases, so that intravenous inoculations should be given if possible in all very bad cases, otherwise intramuscular injections may be employed as a routine. Seeing that excess can do no harm, and if there is any doubt as to the size of the dose, give too much rather than too little, especially with a first dose. Some writers of large experience tell of frequently giving 200,000 units or more in severe cases; there appears, therefore, to be no risk of overdosage. Other writers, however, seem to get equally good results and yet seldom exceed doses of 10,000 units in all; this suggests that no advantage is to be gained by throwing away money on excess of antitoxin. The importance of early administration cannot be over-estimated. If a case is at all suspicious, the administration of 2,000 units should follow *immediately* on the taking of a swab for diagnosis; this is better practice than waiting for the results of the culture. For a definite small patch of membrane confined to one tonsil, seen, say,

for the first time on the second day, 5,000 units is a more suitable dose. If the exudate has spread farther about the fauces give 10,000, and if there are laryngeal symptoms, 15 to 20 thousand units, as an initial dose. So far as age goes, children require proportionately larger doses than adults, as the disease is more fatal in them. Infants may be given 1,000 to 4,000 units, and older children 5,000 to 10,000 units depending on the extent of the lesion and the degree of the toxaemia. Whether more than one dose will be necessary depends again on the individual case. If there is no diminution in the amount of membrane and the general symptoms have not improved the initial dose may be repeated; if the membrane has spread and the toxaemia is more marked, the second dose should be larger than the first. If the symptoms are severe the second dose may be given in from 6 to 12 hours after the first; in less urgent cases the interval may be from 12 to 24 hours, but should not be longer. Injections may be continued at short intervals as circumstances may dictate and in no case need there be any worry as to the total amount given being dangerously large. So long as there is membrane and the patient is toxaemic, then antitoxin should be given.

DYSENTERY (Bacillary)—(*a*) *Vaccines.*—These have not so far come into general use either in the prevention or treatment of Bacillary Dysentery, on account of the difficulty which has been experienced in producing a vaccine, especially of Shiga's bacillus, sufficiently non-toxic to allow of its being inoculated in amounts large enough to be useful. Some have got over this difficulty by using a sensitized vaccine, and with success. A considerable experience in the East African campaign showed that simple broth-cultures of all varieties of dysentery bacilli, killed by carbolic acid, could be inoculated with a degree

of discomfort little or no greater than that following on anti-typhoid inoculation, in amounts sufficient to be of value both in prophylaxis and treatment. It was found, for instance, that a vaccine containing 500 million each of any desired type of *B. dysenteriae* could be given readily; and, indeed, doses of five or even more times that amount were sometimes employed. This work can scarcely be said to have attained common acceptance, but our experience of its worth was sufficient to justify our recommending its employment in appropriate circumstances. In *prophylaxis,* the immunity afforded appears to be of only short duration, a finding which is conformable with the fact that an attack of actual Dysentery confers little immunity; but localized epidemics can be cut short by the inoculation of contacts and those liable to incur infection with one or two doses of vaccine. In *treatment,* the sooner the vaccine can be given the better, and the general experience has been that if one fairly large dose fails to produce a definite improvement it is seldom worth while persevering with it. Both for prophylactic and therapeutic use a vaccine containing the special variety of bacillus causing the trouble, is, of course, the ideal; the identification and preparation of this, however, takes some time, and a stock vaccine containing a number of strains of Shiga's, Flexner's, and Morgan's bacilli is as a rule indicated, at least until a special one more suited to the particular case is obtainable.

(*b*) *Serum.*—Anti-dysenteric serum has been used more extensively than dysenteric vaccines, a multivalent serum being that usually employed. For prophylactic purposes, the use of this serum can only be advised in face of an actual outbreak, the immunity afforded being of such short duration. A subcutaneous inoculation of 20 c.c.s would be appropriate to secure preventive immunity. In treatment, we have learnt that doses considerably larger

than those originally employed are much more likely to be efficacious than peddling with quantities such as ten or twenty c.c.s. If giving serum at all give it boldly, 50 or 100 c.c. intravenously, and repeat on subsequent days if necessary. Attention is directed especially to the remarks on p. 109 as to the keeping of serum. Anti-dysenteric serum seems to be peculiarly sensitive to temperature, and much of the serum used in warm climates is probably quite worthless through lack of appreciation of this detail.

ENTERIC FEVERS (Typhoid, Paratyphoid A, and Paratyphoid B.)—(*a*) *Prophylaxis.*—The incidence of Enteric Fevers in armies, of which they were once the pre-eminent scourge, has fallen enormously since the introduction of antityphoid inoculation. It is now customary to inoculate against Typhoid and the two forms of Paratyphoid Fever at one and the same time by means of a triple vaccine—"T.A.B."—containing the *B. typhosus,* the *B. paratyphosus* "*A*," and the *B. paratyphosus* "*B*." The usual custom is to give two inoculations with an interval of about ten days between them. The first dose contains 500 millions of the *B. typhosus* and half that amount of each of the others; the second dose contains double these amounts. If desired, a third dose of the same strength as the second may be given after a further period of ten days; this is the rule in the U.S. Army. The immunity afforded is of very considerable degree, and lasts as a rule for at least a year. Under special circumstances, anti-dysentery and anti-cholera vaccine may also be added to the inoculum.

(*b*) *Treatment.*—Specific serum treatment has not so far yielded any very striking results in Enteric Fevers. By the use of sensitized vaccines, on the other hand, a shortening of the duration of the disease and a diminution of complications has been

commonly noted. A dose of 50 millions may be given to begin with, followed in 24 or 48 hours by one of 100 millions; subsequently, if the improvement seems to warrant it, even larger doses may be given. When given subcutaneously, little or no general reaction follows on these injections, as a rule. When given intravenously, a sharp reaction may be expected, which may amount even to a termination of the disease by crisis. This appears to be, in reality, an instance of "protein shock" therapy, and, although it cannot be said yet to have become a regular procedure, sufficient has been done to show that valuable and possibly surprising results may follow from the extension of this mode of administering vaccines. As it is naturally to be expected that before commencing vaccine treatment the case will have been definitely diagnosed by cultural or agglutination methods as being one of Typhoid or one or other of the Paratyphoids, the sensitized vaccines are prepared separately for the three organisms, and in any particular case the vaccine of the appropriate bacillus should be used. If this form of treatment should turn out to depend mainly on protein shock it may be that the variety of vaccine used will not matter much, but until this point is more definitely settled it will be advisable to employ the specific vaccine.

GONORRHOEA — *Use of Vaccines.*—In acute urethritis, the doses of gonococcal vaccine used have usually been small—of the order of from 1 to 10 millions; the results obtained, both with simple vaccines and with others prepared by more elaborate methods, have, however, been on the whole so disappointing that most people have given up trying them at this stage. A recent writer (B.M.J., Oct. 6, 1917) reports treatment of a large series of cases with comparatively large doses, and the uniform success he obtained seems to warrant an extended

trial of his methods. The percentage of complete cures obtained was high, the average duration of treatment was short, and the number of cases in which complications occurred (a danger which has been theoretically urged against the use of large doses of vaccines in the acute stage) was less than would have been expected in an equal number of cases treated without vaccines. His routine is to give 50 million gonococci and 150 million staphylococci when the patient is first seen, and double this amount on the third, sixth, ninth, twelfth, and fifteenth days. The other main feature of his treatment is the irrigation of the urethra with weak (1/1,000) permanganate (Janet's system). This course cures many cases; if it does not effect a cure, a second series of inoculations is begun ten days after the cessation of the first, six being given with two days between each, the size of the dose being 100 millions gonococci and 300 millions staphylococci.

Vaccines have a certain field of usefulness in chronic urethritis, but only in combination with other methods of treatment. A stock pure gonococcal vaccine may be used, but as in many of these cases the infection has become a mixed one, a compound gleet vaccine, or an autogenous mixed vaccine, is sometimes preferable. Doses of 250 millions can usually be safely given as a start, but it may be necessary to push the dosage to ten times this amount or more to get satisfactory results.

In the complications of Gonorrhoea, such as arthritis, vaccine treatment has been found much more successful than in the treatment of the original urethritis.

The use of a provocative dose of vaccine as an aid in the diagnosis of doubtful cases has been referred to on p. 27.

HAY-FEVER.—Vaccines prepared from the pollen of grasses have been found very satisfactory in the prevention and cure of Hay-fever in those persons in whom it is definitely due to grass pollen. Various manufacturers prepare outfits which include—(1) a set of test doses with which one can ascertain whether any particular case is sensitive to grass pollen, and also, if the individual is found to be so sensitive, the correct dosage of the vaccine; and, (2) the vaccine in doses of various strengths. As full directions for the employment of the test and actual doses are supplied with the outfits it is unnecessary to go into further detail here.

INFLUENZA.—Although doubt is now being cast upon Pfeiffer's bacillus being the actual cause of Influenza there is no gainsaying its almost invariable association with Influenza in both its acute and subacute forms. On *a priori* grounds, seeing that little or no immunity follows on an attack of Influenza, one would scarcely expect much immunity to follow upon the use of a *B. influenzae* vaccine; it must nevertheless be admitted that the use of such vaccines, containing either the influenza bacillus alone or that organism in combination with other organisms found in various respiratory catarrhal conditions, did appear to be of distinct value in the subacute Influenza or Influenza-like conditions met with prior to the recent epidemic. With regard to the use of vaccines in Epidemic Influenza, which has swept the world recently, no such definite pronouncement can be made. Various vaccines have been tried, including *B. influenzae* alone, *B. influenzae* in association with various strains of pneumococci, and in association with various respiratory catarrhal organisms, or with the particular organisms found to predominate with it locally. A few writers have claimed success for each of these various methods, but the evidence presented has been in the main

merely of the "general impression" order, and on careful analysis it is found that there is no real proof that the vaccines were definitely either of protective or therapeutic value, or that the results obtained by their use were one whit better than those obtained without vaccines. The very most that can be said from the results published with respect to any of these vaccines is that it may have had a slight protective influence. This is essentially the opinion which has also been officially published by the United States Public Health Service, and it is one which from all the evidence available on the use of vaccines in this country, in Europe, and in America, we must at present endorse. We regret that it should be so, but we do not see that any fair-minded person searching after truth irrespective of any other consideration can arrive at any other conclusion. Under pressure at the time of the epidemic we issued a "Compound Influenza Vaccine," but we did so with the expressed proviso that we regarded its employment as purely experimental. We included in it *B. influenzae* from epidemic cases, eight groups of pneumococci, *M. catarrhalis,* and, later, *Streptococcus hæmolyticus*—these being the organisms which we found, and still find, predominating largely in Influenzal Pneumonia. We have had numerous good reports as to the use of this vaccine both as a prophylactic and in treatment.

MALTA FEVER.—This disease, which, if left to itself, may run an almost indefinitely long course, is sometimes very amenable to treatment with a *M. melitensis* vaccine. The dosage employed has usually been about 100 millions, initially, working up to larger doses if occasion seemed to require it.

MENINGITIS (Epidemic Cerebro-spinal or Meningococcal).—Owing to the isolation of the cerebro-spinal fluid from the general circulation one would scarcely expect vaccines to be of great service in the treatment of this disease, and indeed their employment

has been condemned entirely by some observers. There have nevertheless been a fair number of cases recorded in which the use of a vaccine appeared to be of distinct service, and in these days when the employment of vaccines has been found to be of great value in conditions where they would have been looked upon with horror a few years ago, it is unwise to make too sweeping assertions of uselessness on grounds of mere assumption. Doses of from 100 to 1,000 millions have been employed in treatment. A wider scope for meningococcal vaccine at the present time seems to be as a prophylactic in face of an actual epidemic; two doses of 500 and 1,000 millions with a week's interval between them being probably a suitable dose. There may also be a field of usefulness in the treatment of chronic carriers.

Serum treatment offers at present the most hopeful method of therapy in this disease. The serum should be a polyvalent one, or one prepared against the special type of Meningococcus present; at least four of these types have now been definitely recognised. No accurate method of standardizing antimeningococcal serum has yet been devised, and the practitioner must, therefore, be guided by the reputation of the manufacturer as to the value of the serum and his statement as to the amount of it to be used. Subdural inoculation is undoubtedly the best method of administration, and it should be carried out as early as possible in the disease, and with the maximum quantity compatible with safety —the principles referred to on p. 114 being adopted as a guide. Generally speaking, for adults the dose should be about 30 c.c.s, and for young children 15 c.c.s. Remember the importance of very slow injection. It is sometimes recommended that from 30 to 100 c.c.s be also injected intravenously or intramuscularly. It is the usual rule to repeat the subdural injection once a day for the first

four days, and every other day thereafter until acute symptoms subside, and to resume administration if there is any relapse.

PLAGUE.—Vaccines have a very definite use in prophylaxis against Plague. The original Haffkine prophylactic consists of the actual broth with the organisms present therein after five or six weeks' growth. This is still largely employed, but vaccine prepared by washing the bacilli off agar cultures in the ordinary manner appears to be just as efficacious and much less irritating. The latter form of vaccine is the one prepared at the Institute, and as a prophylactic two doses are recommended, one of 500 and the other of 1,000 millions, with a 7 to 10 day interval between them. If only one dose is practicable the larger should be employed. Inoculation does not afford absolute immunity, but it does largely diminish the risk of infection and lower the fatality amongst those attacked. The immunity afforded, however, is not of long duration, possibly not over three months and almost certainly not for more than six.

PNEUMONIA.—Although many organisms may be found in the sputum of pneumonic patients, as in that of others, the work which Lister has carried out at the Institute has shown that Lobar Pneumonia in South Africa is essentially a purely pneumococcal infection. It has shown, moreover, that pneumococci, although morphologically and culturally all practically alike, can be sub-divided by immunological methods into a number of distinct groups or types. These types "breed true" apparently indefinitely, and are to all intents and purposes separate species. A number of these have now been identified not only here but also in other parts of the world, and the importance of their recognition is being generally realised in the war against Pneumonia, that "Captain of the men of Death"—to use

an expression which is even more justified in Africa than in the circumstances in which it was first employed. The importance of the recognition of these different groups of pneumococci lies in the fact that whilst inoculation with any one group brings about a condition of immunity against that group it gives little or no protection against other groups. Similarly in treatment—early inoculation of a case of pneumococcal pneumonia with a vaccine of the group responsible for the infection often produces brilliant results, whilst a vaccine of one of the other groups would be without effect. The total number of definitely distinct groups of pneumococci is unknown, but Lister has identified at least ten, which between them appear to be responsible for about 90% of the cases of lobar pneumonia occurring in South Africa. Two of these ten are rare, and of the remaining eight some are numerically much more important than others. It is noteworthy that the incidence of infections by the various groups is different in Europeans and dark-skinned races. Some groups are more commonly found in the pneumonias of white people than in those of coloured persons, and *vice versa,* although no race is entirely immune to any of the identified groups.

It is the custom of vaccine manufacturers to describe all the cultures of any organism which have been derived originally *from different patients* as so many different " strains " of the organism. It should, therefore, be realized that a vaccine which is advertised as containing a very large number of different " strains " of pneumococcus may, in reality, only contain one of the essentially important groups or types. Such a vaccine would be of service, of course, only against the particular group it contained; the fact of there being a large number of different " strains " present would confer no particular advantage.

Vaccines in prophylaxis.—The experimental work of Lister here, and the employment of pneumococcal vaccines on a large scale locally and in America, have abundantly proved that an adequate degree of protection against Pneumonia can be obtained by prophylactic immunization. To secure this, however, massive doses must be given. The practice recommended by us at the present time is to inoculate three times at weekly intervals with 1 c.c. of Lister's 8-group pneumococcal vaccine, each 1 c.c. of which contains 1,000 millions of each of the eight common groups, *i.e.*, 8,000 millions of pneumococci in all. This may sound very heroic dosage, but an experience which now runs into some millions of inoculations has not shown any consequent ill-effects. A certain number of individuals do, it is true, have a rather intense local and general reaction (usually only after the first inoculation), but their number is not very great and even then the reaction is not of long duration. To meet the demands for this vaccine on the mines of the Witwatersrand, demands which are alone equivalent to nearly 800,000 doses a year, an elaborate and extensive plant has been developed at the Institute. It is now possible to turn out the vaccine in enormous quantities with the same meticulous attention to securing sterility and absence of contamination that is devoted, for instance, to the preparation of an individual autogenous vaccine. Improvements in technique have now enabled us also to prepare this vaccine without the addition of any more preservative than is customarily added to all vaccines. Recent work in America has shown that it is practicable to give even still larger doses, and to administer the dose in such a manner that one inoculation will produce the desired immunity. If this could be done on a large scale it would certainly be a great advantage, and the next step that we are looking forward to is to make this practicable.

The duration of the immunity given by the three inoculations at present recommended is probably for not less than one year and possibly for considerably longer.

Vaccines in treatment.—The use of vaccines in the treatment of Lobar Pneumonia, although still said by some writers to be theoretically unsound, has been shown by actual trial often to lead to the most happy results, and has not, in our experience, ever given rise to any bad effects. It is probably of little service to attempt vaccine therapy at a late stage of the disease, but we believe that if it is tried early the results will often be found to be most satisfactory. In view of the specificity of the different groups of pneumococci the ideal vaccine is, of course, one of the particular group infecting the patient, but, as it takes from 24 to 48 hours to isolate and identify this, Lister's 8-group pneumococcal vaccine should be given while the type of the patient's infection is being identified. An autogenous vaccine, or one of the patient's particular type, may be employed later. It is recommended that a dose of at least 8,000 millions of the appropriate stock vaccine be administered at the earliest possible stage of the disease; this may be repeated 48 hours later if a vaccine of the special type affecting the patient has not, in the meantime, become available.

Serum Treatment.—Recent work at the Rockefeller Institute has demonstrated that treatment of Pneumonia by means of anti-pneumococcal serum will probably be *the* treatment of choice in the future. The serum must, however, be one prepared against the homologous type of pneumococcus or else a multivalent one, and such sera are not as yet available in quantity.

OTHER PNEUMOCOCCAL AFFECTIONS.—Little can be said about the use of vaccines or sera in other pneumococcal conditions (such as Pericarditis, Meningitis, joint affections, etc.), but judging from the results obtained in Pneumonia they would probably be worth trial in all cases definitely shown to be pneumococcal in origin.

PUERPERAL SEPTICAEMIA.—About 80% of these cases appear to be streptococcal in origin. Anti-streptococcal serum has, however, been very disappointing in these cases (possibly largely because of the inefficiency of the serum used) and the best results have been obtained by the use of vaccines, in addition, of course, to ordinary surgical procedures. A stock vaccine prepared from streptococci of puerperal origin should be used as early as possible and whilst the bacteriology of the particular case is being investigated and an autogenous vaccine prepared. An initial dose of 25 millions will usually be found satisfactory, to be followed in 24 hours, if there are no contrary indications, by one of double that amount. Still larger doses may be subsequently employed, their administration being regulated by the rules governing vaccine therapy in general.

PYORRHOEA ALVEOLARIS.—Vaccine treatment has frequently been found to be of service in this condition, but only as an adjunct to dental measures; to attempt to cure a case by vaccine therapy alone is simply inviting failure. Either a stock Pyorrhoea vaccine prepared from a number of the organisms commonly found in this condition (Streptococci, M. catarrhalis, etc.) or an autogenous vaccine may be used; but to obtain good results it is usually necessary to continue the treatment for a considerable period and to push the dosage to fairly high amounts.

RHEUMATISM.—Vaccines cannot be said to produce consistently good results in the treatment of rheumatic affections, but some cases do undoubtedly respond to their employment; the particular cases which will benefit can, unfortunately, only be found out by trial. A stock *Streptococcus rheumaticus* vaccine can be employed more frequently than an autogenous one. It is well to begin with small doses, say, 25 millions, in both acute and chronic conditions, since the reaction after inoculation is apt, if too large doses be employed at first, to be rather severe. In the more chronic cases fairly large doses may be subsequently worked up to with advantage.

SNAKE-BITE.—The toxins of snake venoms are soluble albumoses the action of which is mainly either on the nerve cells (neurotoxins) or on the blood and blood vessels (haemolysins and haemorrhagins). Specific treatment by antivenene is the only thing that is of any service after venom has been absorbed, and even then it has to be used quickly if it is to be of service. Unfortunately also antivenenes are specified in their action and not general, *i.e.*, they neutralize only the venom of the species against which they have been prepared, not the venom of a different species of snake. Even the best of them is comparatively weak, and large doses are necessary, not less than 100 c.c.s (preferably intravenously) being the least that is likely to be of use. Antivenene may now be obtained from the Institute.

TETANUS.—The value of Tetanus antitoxin may be summed up in the statement that as a prophylactic it exceeds diphtheria antitoxin in value, whilst it is far inferior to it as a therapeutic agent; the reason being that tetanus toxin unites so quickly and so firmly with the nerve-cells of the spinal cord. Toxin may be produced, and its absorption

may commence, within a day or two of infection, but its production may be delayed for some weeks. Antitoxin in the circulation will neutralize the toxin as quickly as it is formed, hence the importance of a prophylactic inoculation of antitoxin as soon as possible after an injury with which there is a likelihood of there being a Tetanus infection. An intramuscular injection of 1,500 units, given near the wound so as to flood the tissues in its immediate vicinity, is sound prophylactic practice. If the patient is not seen until a few days after the injury has been inflicted a 5,000-unit intravenous injection, for the immediate neutralization of any toxin that may be in the blood, is recommended. For efficient prophylaxis, antitoxin should be present in the circulation for at least two or three weeks, and to maintain this condition a second dose of about 1,000 units should be given from 7 to 10 days after the first. All antitoxin prophylaxis is, of course, recommended only as an addition to the usual surgical treatment.

In the treatment of cases in which tetanic symptoms have shown themselves antitoxin is admittedly less successful; it nevertheless still offers the patient more hope than any other measure. The aim is to neutralize all toxin in, or entering into, the circulation, and also, as far as possible, to dissociate and neutralize the toxin which has already reached and entered into combination with the nerve-cells. To attempt this with reasonable hope of success large doses and rapid action of the antitoxin is necessary, and it is, therefore, advised that 10,000 to 20,000 units should be given intravenously and 3,000 to 5,000 units subdurally. These doses should be repeated in 24 hours unless the symptoms have greatly ameliorated, in which case intramuscular injections of from 5,000 to 10,000 units will probably be sufficient to maintain the good effect.

TUBERCULOSIS.—The value of tuberculin in the treatment of Tuberculosis is such a debateable subject, the varieties of "tuberculin" are so many, and their use is admittedly so dangerous unless employed with the greatest circumspection, that it is impossible briefly to discuss this subject here. Anyone wishing to use tuberculin, unless already fully *au fait* with the subject, is therefore advised to consult some of the works dealing fully with the subject.

ULCERS AND VELD SORES.—Most of these are staphylococcal in origin, and, in obstinate cases which will not heal up under ordinary measures, a vaccine is frequently of the greatest benefit. A stock staphylococcal vaccine may be used or an autogenous one may be preferred, more especially if an examination of smears shows that organisms other than staphylococci are present. Fairly large doses can usually be administered with safety and advantage.

WHOOPING COUGH.—A *B. pertussis* vaccine has been employed with success both in the prophylaxis and treatment of Whooping-cough. For the prophylactic inoculation of contacts the following doses may be employed:—for infants, 100 millions; for older children, 250 millions. In treatment, it has been found that the violence of the paroxysms is lessened and the duration of the illness considerably shortened. A suitable initial dose is, for infants, 50 millions, and for older children, 100 millions. Further doses may be given at intervals of from 4 to 7 days, the dosage being increased or not as considered advisable from the effects of the first dose.

LABORATORY No. 15.

APPENDIX I.

Tariff of Charges for Investigations.

(Outfits for the collection of Specimens will be sent free on application. A list of outfits will be found on pages 56 and 57).

	£	s.	d.
BLOOD.			
Microscopic examination of Films for malarial or other parasites	0	10	6
Microscopic examination with differential leucocyte count	1	1	0
Quantitative estimation of haemoglobin and erythrocytes	1	1	0
Total and Differential leucocyte count ...	1	1	0
Complete blood count	2	2	0
Spectroscopic examination of blood ...	1	1	0
Wassermann test	1	1	0
Agglutination (Widal) test—for Typhoid and Paratyphoid Fevers	0	10	6
Agglutination test for Malta fever or other conditions	0	10	6
Cultural examination for micro-organisms	1	1	0
CEREBRO-SPINAL FLUID.			
Wassermann test	1	1	0
Microscopic examination for cells and organisms	0	10	6
Cultural examination for micro-organisms	1	1	0
Cell count	0	10	6
Chemical examination for Globulin, etc.	0	10	6
DISINFECTANTS.			
Determination of Rideal Walker co-efficient ... ... from	5	5	0
FAECES.			
General miscroscopic examination for blood, pus, ova, amoebae, or other parasites ... ... from	0	10	6

	£	s.	d.
Chemical examination for occult blood	0	10	6
Isolation and identification of special organisms such as B. typhosus, B. dysenteriae, etc.	2	2	0
Microscopic examination for B. tuberculosis by Antiformin method	0	10	6

FOOD-STUFFS.

General bacteriological examination from	3	3	0

GASTRIC CONTENTS.

Microscopic examination	0	10	6
Quantitative estimation of free and combined acids	1	1	0

HAIR OR EPIDERMAL SCALES.

Examination for parasitic fungi, etc. ...	0	10	6

MILK.

General bacteriological examination ...	3	3	0
Microscopic examination for presence of B. tuberculosis	1	1	0
Ditto, with biological test	2	2	0
Isolation of special organisms, *e.g.*, B. diphtheriae or B. typhosus	4	4	0

PUS, EXUDATES, DISCHARGES, BODY FLUIDS, ETC.

General microscopic examination for cells, micro-organisms, etc.	0	10	6
General microscopic examination with cultural examination	1	1	0
General microscopic examination with biological test	2	2	0
Direct examination for T. pallida ...	0	10	6
Cultural examination for B. diphtheriae only	0	10	6

	£	s.	d.
SEWAGE.			
General bacteriological examination from	5	5	0
SPUTUM.			
Microscopic examination for B. tuberculosis	0	7	6
Ditto, with report on other organisms present	0	10	6
Examination for B. tuberculosis by Antiformin method	0	10	6
Ditto, with Biological test	1	11	6
General microscopic and cultural examination including examination for B. tuberculosis	1	1	0
TUMOURS, DISEASED TISSUES, ETC.			
Histological examination and report with one stained section, if desired ...	1	1	0
Extra stained sections ... ... each	0	2	6
Pathological specimens preserved and mounted ... ... from	0	10	6
Post-mortem examinations conducted at Institute Mortuary, with report from	2	2	0
URINE.			
General examination; *i.e.*, specific gravity, reaction, presence or absence of albumen and glucose, microscopic examination of centrifugalized deposit	0	10	6
General examination with quantitative estimation of any one of the following, glucose, urea, or albumen	0	15	0
Any other special features, extra from	0	5	0
Cultural examination for organisms ...	1	1	0
Examination for B. tuberculosis	0	10	6
Ditto, with Biological test	1	11	6
Examination for Bilharzia	0	10	6

	£	s.	d.
VACCINES.			
Autogenous, Isolation of infecting organisms and preparation of. In rubber capped bottle containing sufficient for a course of treatment	2	2	0
Autogenous sensitized vaccines ... from	5	5	0
Vaccines (Stock). See Appendix II.			
WATER.			
General bacteriological examination ...	3	3	0
Test for B. coli only	1	11	6
MISCELLANEOUS.			
Sterilizing apparatus, testing efficiency of	1	1	0
Testing sterility of surgical dressings each	0	10	6

APPENDIX II.

List of Bacterial Vaccines, Sera, etc.,

Prepared in the Routine Division of the Institute.

BACTERIAL VACCINES.

Owing to the very large numbers of specimens handled yearly, the Institute possesses exceptional facilities for obtaining typical and atypical strains of the various micro-organisms needed for the preparation of its vaccines; new types and fresh strains are being constantly added to improve their efficiency.

These vaccines are **polyvalent,** are prepared from **South African strains,** are **accurately standardised,** and are of **assured sterility.**

Ordinarily not more than five concentrations of a vaccine are kept ready packed, but **any concentration other than those mentioned in this list can be supplied at short notice.**

Owing to the growing preference for increased dosage in vaccine therapy, the lower strengths hitherto listed have been discarded, but, where smaller doses than the initial ones recommended herein are preferred, the appropriate proportion of the lowest stock concentration should be used. Similarly, where intermediate doses differing from those listed are required, an appropriate lower volume of the next higher listed concentration will furnish the requisite dose.

For information as to the general principles governing the use and dosage of vaccines, see page 97 *et seq.*

Practitioners who make frequent use of stock vaccines will find it highly advantageous to order these in 25 c.c. rubber-capped bottles, for which special prices will be quoted.

General Directions for Use of Vaccines.

The Site for Inoculation.—The injection is preferably made into the subcutaneous tissues; the best sites for the purpose are (1) in the flank, (2) from 1½ to 2 inches below the centre of the clavicle, or (3) the outer surface of the upper arm.

Preparation of the Skin.—Disinfect the skin at the area selected with Tincture of Iodine.

Withdrawing the Vaccine.—(a) **From an ampoule:** Sterilize a hypodermic syringe by boiling, and affix the needle. Shake the ampoule vigorously for a few seconds, then give it a sharp downward jerk in order to dislodge any fluid remaining in the stem. Cover the stem with a pad of cotton-wool or lint and grasp it between the thumbs and forefingers with the thumb-nails close to one another; a slight bending strain will now cause the stem to snap. Any fragments of glass that may splinter off will remain in the pad of wool or lint. The broken ampoule should now be inverted over the needle and the desired volume of vaccine drawn into the barrel of the syringe. (b) **From a rubber-capped bottle:** Similarly sterilize a syringe and affix the needle. Shake up the contents of the bottle. Place a drop of Lysol, Tincture of Iodine or other strong disinfectant on the cap. Thrust the needle through the disinfectant and cap into the vaccine, reversing the bottle if need be, and draw up the volume required. To close the opening wipe off the disinfectant and seal the puncture by lightly touching the wax coating with a burning match or hot wire.

Injecting the Vaccine.—Grasp a fold of skin between the thumb and forefinger of one hand, holding the syringe in the other, boldly plunge the needle through the skin, gradually inject the fluid, then quickly withdraw the needle.

Stains of Iodine may be removed from the skin, if desired, by washing with solution of Hyposulphite of Soda ("Hypo.").

Acne (Compound) Vaccine.

(*Bacillus acnes and Staphylococcus*).

In order to render this vaccine suitable for practically every case of pustular acne it has been compounded from a large number of strains both of the Acne bacillus and of Staphylococci (albus, aureus, citreus, etc.).

Acne

Suggested initial dose = 100 million organisms

Interval between doses = one week.

Concentration (Millions per c.c.)	100	250	500	1,000	2,000
Price per dose	2/-	2/6	3/-	3/6	4/-

Bacillus Coli Vaccine.

This vaccine is prepared from many strains of the Bacillus coli and other representatives of the Coliform group of bacilli. It may be employed in most conditions in which such organisms are present, e.g., Appendicitis, Cystitis, Pelvic abscess, etc.

Appendicitis.
Cystitis.
Pelvic Abscess

Suggested initial dose = 100 million organisms

Interval between doses = one week.

Concentration (Millions per c.c.)	100	250	500	1,000	2,000
Price per dose	2/-	2/6	3/-	3/6	4/-

Compound Coryza (Catarrhal) Vaccine.

This vaccine has proved of distinct value—both curative and prophylactic—in the treatment of Recurrent Colds, Naso-pharyngeal Catarrhs, Influenzal attacks, etc. It may also be found useful in Bronchitis, Septic Sore Throat and Asthma; in these affections, however, autogenous vaccines are usually superior and should be employed if possible.

Catarrh.
Septic Throat.
Influenza.
Bronchitis.

This vaccine contains many strains of Streptococcus, Micrococcus catarrhalis, Pneumococcus, Friedlander's bacillus, Bacillus septus, Bacillus influenzæ, etc. The preparation is varied from time to time to suit the prevailing types of infection, and is put up in five graduated strengths (Nos. 1 to 5). Treatment should be commenced with a "No. 1" dose, and, in the absence of any indications to the contrary, continued with doses "2" to "5" at intervals of 3 to 5 days. About 3 months later further injections of Nos. "4" and "5" should be administered in order to reinforce the immunity.

Numbers	1	2	3	4	5
Price per dose	2/-	2/6	3/-	3/6	4/-

Gleet Vaccine.

Gleet.

In chronic Gleet, organisms other than the Gonococcus appear often to be responsible for keeping up the discharge, especially Staphylococci.

The infection may be a mixed one of various organisms and an autogenous vaccine is probably better in many cases, but this vaccine, containing a number of strains of Staphylococci of urethral origin, together with small proportions of Streptococci and Gonococci, will be found useful in a variety of cases. If Gonococci are still obviously present in the discharge it is advisable to combine the use of a pure Gonococcal vaccine with this.

Suggested initial dose = 250 million organisms
Intervals = 4—7 days.

Concentration (Millions per c.c.)	250	500	1,000	2,000	4,000
Price per dose	2/6	3/-	3/6	4/	5/-

Gonorrhœa Vaccine.

(*Micrococcus gonorrhœa*).

Gonorrhœa.

This vaccine is often of great assistance in clearing up a gonorrhœal infection. In acute and sub-acute forms of the disease treatment may be commenced with small doses, e.g., 10—50 million organisms. Intervals of 3—5 days should be allowed between the first three or four doses, later 7—10 days.

In more chronic cases the 100—250 million strength may be used to start with.

Concentration (Millions per c.c.)	100	250	500	1,000	2,000
Price per dose	2/-	2/6	3/-	3/6	4/-

Influenza (Compound) Vaccine.

Influenza.

This mixed vaccine contains the Bacillus influenzæ, Micrococcus Catarrhalis, and Streptococci, together with 8 Pneumococcal Groups, and comprises the most common organisms which were found locally to be prevalent during the 1918 epidemic of influenza.

It may be used for both prophylactic and therapeutic purposes

A pure B. Influenza Vaccine can be supplied, if preferred, for the same purpose, but, unless this is specifically asked for, the compound vaccine will be furnished.

The compound vaccine is supplied in one strength only, and the initial dose recommended for both preventive and curative purposes is 1 c.c.

By arrangement with the Union Department of Public Health this vaccine is supplied to anyone for use within the Union at the nominal price of 9d. per single dose.

Special prices for bulk quantities in rubber-capped bottles of 25. 50 and 100 c.c. on application.

Malta Fever Vaccine

(*Micrococcus melitensis*).

This disease, which is usually difficult to treat with success by the usual methods, is sometimes amenable to vaccine therapy. **Mediterranean or Malta Fever.**

Suggested initial dose = 100 million.
Intervals = 1 week.

Concentration (Millions per c.c.)	100	250	500	1,000	2,000
Price per dose	2/-	2/6	3/-	3/6	4/-

Meningitis Vaccine.

(*D. intracellularis meningitidis*).

Vaccines of this organism have been employed as a prophylactic and, to some extent, in treatment, both of carriers and of acute cases of cerebro-spinal meningitis. Probably they are of most value for inoculating those who have been in contact with acute cases. For the treatment of acute cases the specific serum should be used. **Cerebro-Spinal Meningitis.**

Two or three inoculations of the vaccine, at intervals of a week, working up to a final dose of 1,000 millions, have been recommended.

Concentration (Millions per c.c.)	100	250	500	1,000
Price per dose	2/-	2/6	3/-	3/6

Plague Vaccine

(*Bacillus pestis*).

In the preparation of this vaccine the bacillus is grown upon the surface of a solid medium; the resulting vaccine is therefore less toxic and gives rise to less severe reactions than that of Haffkine. **Plague.**

In the event of an outbreak of Plague all individuals within the threatened area should receive two injections, of 500 million and 1,000 million organisms respectively, at intervals of 7 to 10 days.

Concentration (Millions per c.c.) 500 1,000

Special prices for bulk quantities in rubber-capped bottles of 25, 50, 100 or 200 c.c. on application.

Pneumococcal Vaccine (Lister's 8-Group).

Pneumonia.

This vaccine—as used with marked success on the mines of the Witwatersrand and elsewhere for the prevention and cure of Pneumonia—is prepared in accordance with the recent researches of Dr. F. S. Lister, of this Institute, and contains identified representatives of those **immunologically distinct groups of the Pneumococcus** which are responsible for about 90 per cent. of the cases of Lobar Pneumonia occurring in Europeans.

Prophylactic Inoculation.—Experiments performed upon a large scale have proved conclusively that, in order to secure adequate immunization, massive doses must be given and repeated Satisfactory results have been obtained by injecting **8,000 million** dead organisms upon each of three occasions, the interval between each dose being one week.

Therapeutic Inoculation.—At the earliest possible stage of the disease a dose of at least **8,000 million** organisms may be administered, and this dose should be repeated once or twice at intervals of 48 hours.

It is probably of little service to attempt vaccine therapy at a late stage of the disease; in the early days of an attack, however, the treatment has been attended with much success in many cases.

The employment of these comparatively large doses, both in preventive and curative treatment, has not—in an experience of over 2,000,000 inoculations—given rise to any ill effects, although in some individuals who are particularly sensitive a fairly severe reaction may occur.

Concentration—8,000 millions per c.c. (1,000 millions of each of eight classified groups).

Price per series of three ampoules, 10/-.

Special prices for bulk quantities in rubber-capped bottles of 25, 50, 100 or 200 c.c. on application.

Puerperal Vaccine

(*Puerperal Streptococcus*).

Many cases of Puerperal Septicæmia have been treated with this vaccine with great success. An injection of 25 million organisms should be given at the earliest possible moment, followed after 24 hours by one of 50 million, and after a further interval of 48 hours by doses of 100 million and 250 million organisms respectively.

Puerperal Septicæmia

Concentration (Millions per c.c.)	25	50	100	250
Price per dose	2/-	2/6	3/-	3/6

Pyogenic Vaccine

(*Streptococcus and Staphylococcus*).

This vaccine is intended for use in conditions in which both Streptococci and Staphylococci are commonly concerned as causal agents, such as Septic Wounds, Abscesses, etc.

Septic Wounds. Abcesses.

The dosage is dependant, of course, upon the severity of the symptoms, but a dose of 100 millions will generally be found to be a suitable one to commence with; should, however, the patient be running a high temperature a smaller dose, e.g, 25—50 millions would be safer. Intervals 5–10 days.

Concentration (Millions per c.c.)	100	250	500	1,000	2,000
Price per dose	2/-	2/6	3/-	3/6	4/-

Pyorrhœa Vaccine.

This vaccine is composed of several strains of the various micro-organisms commonly found in cases of Pyorrhœa alveolaris such as Streptococcus, Staphylococcus, Micrococcus catarrhalis, etc. It may often be found useful in clearing up an obstinate Pyorrhœa; in some cases, however, treatment must be carried out over a period of several weeks or months before the optimum effect is obtained.

Pyorrhœa.

Suggested initial dose = 50 million organisms. Intervals = 1 week.

Concentration (Millions per c.c.)	100	250	500	1,000	2,000
Price per dose	2/-	2/6	3/-	3/6	4/-

Rheumatism Vaccine

(*Streptococcus rheumaticus*).

This vaccine is prepared from strains of Streptococcus which have been recovered from cases of Acute Rheumatism. In cases of this nature, as well as those of Malignant Endocarditis, vaccine treatment should be initiated as early as possible.

Rheumatism. Endocarditis

Suggested initial dose = 100 million organisms. Interval = 4—7 days.

Concentration (Millions per c.c.)	100	250	500	1,000	2,000
Price per dose	2/-	2/6	3/-	3/6	4/-

Staphylococcus Vaccine.

This vaccine is of curative value in the treatment of Boils, Carbuncles, Abscesses, and other Staphylococcic infections.

Carbuncles.

Boils, etc.

Suggested initial dose = 100—500 million organisms. Interval = 1 week.

Concentration (Millions per c.c.)	100	250	500	1,000	2,000
Price per dose	2/-	2/6	3/-	3/6	4/-

Streptococcus Vaccine.

This vaccine is employed usually with beneficial results in Erysipelas, Septicæmia and Peritonitis; it may also be used with success in certain cases of Otitis Media, Appendicitis and Abscess.

Septicæmia, etc.

The initial dose should not exceed 50 million organisms, while in very acute conditions a dose of 25 millions is preferable.

Intervals = 4—7 days.

Concentration (Millions per c.c.)	100	250	500	1,000	2,000
Price per dose	2/-	2/6	3/-	3/6	4/-

Typhoid-Paratyphoid ("T.A.B.") Vaccine.

(*Bacillus typhosus, Bacillus paratyphosus "A." Bacillus paratyphosus "B."*)

This vaccine is supplied in sets of two immunizing doses, the first containing 500 millions of B. typhosus, 250 millions of B paratyphosus (A), and 250 millions of B. paratyphosus (B), and the second containing twice these numbers of the respective bacilli.

Typhoid Prevention.

It is recommended that an adult should be given 1,000 millions of the combined bacilli for the first injection, and double that number ten days afterwards.

When ordering this vaccine it is desirable to state how many first and how many second doses are required.

By arrangement with the Union Department of Public Health this vaccine is supplied to anyone for use within the Union at the nominal price of 1/- per set of two doses.

Special prices for bulk quantities in rubber-capped bottles of 25, 50, 100 or 200 c.c. on application.

Typhoid or Paratyphoid Vaccine

(*Sensitized*).

Sensitized vaccine has yielded good results in the treatment of Enteric fevers; often bringing down the temperature to normal limits within a few hours of the first or second injections. It is preferably administered **intravenously.**

Treatment of Enteric Fevers.

An initial dose of 50 million organisms should be followed by one of 100 millions after an interval of 24 to 48 hours, and subsequently, after similar intervals, by 250 and 500 million. In many cases the inoculation produces a rapid rise of 1 or 2 degrees in temperature; this, however, soon subsides and is followed by substantial improvement in the patient's condition. Various observers have stated that the occurrence of dangerous sequelæ is considerably reduced by the use of Sensitized Vaccines.

Naturally, before treatment with a sensitized vaccine is commenced, the case will be diagnosed by cultural or agglutination methods as being due either to the B. typhosus, the B. paratyphosus "A," or the B. paratyphosus "B." Stocks of these are kept separately and, when ordering, it should be stated which one is desired. When this is not specifically done, a sensitized B. typhosus vaccine will be supplied.

Concentration (Millions per c.c.)	50	100	250	500
Price per dose	5/-	7/6	9/6	10/6

Whooping Cough Vaccine

(*Bacillus pertussis*).

It has recently been shown that Whooping Cough responds to vaccine treatment provided that suitable doses are employed. By this means the violence of the paroxysms is lessened and the duration of illness considerably curtailed.

Pertussis.

Suggested initial dose for infants = 50 million organisms; for older children = 100 million. Interval = 4 to 7 days.

Concentration (Millions per c.c.)	50	100	250	500
Price per dose	2/-	2/-	2/6	3/-

OTHER PRODUCTS OF THE DEPARTMENT INCLUDE THE FOLLOWING :—

Anti-Dysenteric Serum

(*Polyvalent*).

This serum is prepared from many strains of Dysentery bacilli (both "Flexner" and "Shiga" types) isolated from cases occurring in South and East Africa. It is of high titre, sterile and non-toxic.

Dosage.—In cases of bacillary dysentery, 50 c.c. should be given twice daily, and in very severe cases four times daily, for two or three days. The injections may be made under the skin of the abdomen or flank, but are preferably given intravenously.

Price per dose, 7/6.

Normal Horse Serum.

This serum may be administered (1) **hypodermically** in cases of Simple Anæmia, Purpura Hæmorrhagica, etc.; (2) **orally** in Hæmoptysis, Hæmophilia, Hæmatemesis, Gastric and Duodenal Ulcer, etc.; (3) **as a local application** to chronic suppurating wounds or indolent ulcers.

Note.—When inoculating hypodermically it is advisable to inject one or two minims first, and the remainder after an hour or so, provided that no symptoms of anaphylaxis have developed in the interval.

Price per dose: 10 c.c. = 2/-; 25 cc. = 3/6; 50 c.c. = 6/-.

Normal Ox or Goat Serum.

Either of these may be used instead of Normal Horse Serum. They may be employed in the same manner and for the same purposes. They are specially advised for cases known to have been treated previously with any serum of equine origin, such as Diphtheria Anti-toxin, Anti-Streptococcic Serum, etc. The possibility of anaphylactic symptoms supervening is thus obviated.

Normal Ox Serum has also recently been recommended in the treatment of Anthrax.

Price per dose: 10 c.c. = 2/-; 25 c.c. = 3/6; 50 c.c. = 6/-.

Bulgarian Milk Culture.

This culture is a descendant of one which was originally isolated in Bulgaria. It contains special Lactic-acid-producing bacteria and is used as a "starter" for the preparation of soured milk. It is prescribed for the treatment of various gastric and intestinal disorders, and is of great benefit to poorly nourished infants and children. Directions for use accompany each bottle.

Price per bottle, 2/6; by post, 2/9.

OTHER SERA AND VACCINES STOCKED BY THE INSTITUTE.

Owing to the difficulty frequently experienced in obtaining various Sera, not at present produced in South Africa, the Institute now carries stocks of the following, prepared in overseas laboratories. These will be supplied at usual current prices:—

Anti-Diphtheria Serum, 2,000, 4,000 and 8,000 Units.
Anti-Meningitis Serum.
Anti-Streptococcus Serum (Polyvalent).
Anti-Tetanus Serum.
Antivenene (for Snake-bite).
Pollaccine (for Hay Fever).
Hay Fever Reaction Outfits.

In each case directions for use accompany the product.

Other products will be added to the above list as occasion arises.

Calf Vaccine Lymph, prepared at the Government Vaccine Station, Rosebank, Capetown, may also be obtained from the Institute (free of charge) by medical practitioners and local health authorities in the Union. When, however, large quantities are required they should be requisitioned for direct from the Government Bacteriologist, Capetown.

Upper West Corridor.

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